# 2nd ASM Conference on Prokaryotic Development

Washington, DC 20036-2904

Phone: 202-737-3600

World Wide Web: www.asm.org



Printed in the United States of America

ISBN: 1-55581-350-X

# TABLE OF CONTENTS

## ASM CONFERENCES COMMITTEE

**Lawrence Shimkets, Chair**
University of Georgia

**Robert LaRossa, Vice Chair**
DuPont de Nemours & Company

**Bonnie L. Bassler**
Princeton University

**William Goldman**
Washington University School of Medicine

**Jo Handelsman**
University of Wisconsin

**Ann Hill**
Oregan Health & Science University

**Virginia Miller**
Washington University School of Medicine

**Lance R. Peterson**
Northwestern University

## ASM CONFERENCES MISSION

To identify emerging or underrepresented topics of broad scientific significance.

To facilitate interactive exchange in meetings of 100 to 700 people.

Encourage student and postdoctoral participation.

To recruit individuals in disciplines not already involved in ASM to ASM membership.

To foster interdisciplinary and international exchange and collaboration with other scientific organizations.

## SCIENTIFIC PROGRAM ORGANIZERS

**Jeff Errington**

Oxford University, Oxford, UK

**Heidi Kaplan**

University of Texas, Houstin, TX

## ADVISORY COMMITTEE

**Mark Buttner**

**Yves Brun**

**Susan Golden**

**Kit Pogliano**

**Lotte Sogaard-Anderson**

## ACKNOWLEDGMENTS

The American Society for Microbiology and the scientific program organizers would like to acknowledge the following for their contributions to this conference:

Prolysis Corporation

Society for General Microbiology (SGM)

U.S. Department of Energy (DOE)

## REGISTRATION

On July 13, the registration desk will be located in the Walter Gage Residence Hall. On subsequent days, the ASM staff will be located in in the Curtis Law Building, room 179/181, to assist participants during session hours.

## GENERAL SESSIONS

All general sessions will be held in the Curtis Law Building, room 101/102/201. A name badge is required for entry into all sessions. In consideration of other participants, no children are permitted in the sessions. Every seat must be occupied. Please be considerate of fellow participants and fill in every seat.

## POSTER SESSIONS & COFFEE BREAKS

Poster boards are located in the foyer of the Curtis Law Building. Posters for session A should be mounted Wednesday evening before the opening session and should be removed by no later than the conclusion of the evening session on Thursday. Posters for session B should be mounted before the morning session on Friday, and should be removed no later than the conclusion of the morning session on Saturday. Each poster is allotted half of a board space. Please check your assigned number in the abstract index and mount your poster on the board space bearing that number.

Official poster sessions will be held on Thursday and Friday as noted within the program. Poster presenters whose abstracts are designated with an " A " should be at their poster board during the official poster session on Thursday; presenters for "B" session should be at their poster board during the official poster session on Friday.

## MEALS

The opening reception, breakfasts on Thursday, Friday, and Saturday, and dinners on Thursday and Friday will be held in the First Nations Longhouse, a short walk from the Gage Residence and the Curtis Law Building. The conference dinner on Saturday will be held at the Museum of Anthropology, also on campus within walking distance of the meeting facilities. Lunches are not included in the registration fee. There are many varied options for lunchtime dining on your own on campus.

# WEDNESDAY JULY 13TH

**Session 1: Cell Cycle**
Session Chair: Liz Harry

**6:00 – 6:10 pm** **Welcome Remarks**

**6:10 – 6:40 pm** *Caulobacter* Cell Division and Segregation
**Lucy Shapiro**, Stanford University, Stanford, CA, USA

**6:40 – 7:10 pm** SlmA, a Nucleoid-associated Protein that Coordinates Cytokinesis with Chromosome Positioning in *E.coli*
**Piet De Boer**, Case Western Reserve University, Cleveland, OH, USA

**7:10 – 7:30 pm** Positional Control of Replication in *Bacillus subtilis*
**Melanie B. Berkmen**, Massachusetts Institute of Technology, Cambridge, MA, USA

**7:30 – 7:50 pm** Coordination of Chromosome Partitioning and Cytokinesis in *Caulobacter*
**James W. Gober**, UCLA, Los Angeles, CA, USA

**7:50 – 8:10 pm** Multiple Interactions between the Transmembrane Division Proteins and Cell Elongation Protein of *B. subtilis*
**Richard Daniel**, Univ. of Oxford, Oxford, UNITED KINGDOM

**8:15 – 10:00 pm** **Reception**

# THURSDAY JULY 14TH

**7:00 – 8:15 am** **Breakfast**

**Session 2: Cytoskeleton**

Session Chair: Larry Rothfield

**8:30 – 9:00 am** Cytoskeleton and Cell Shape in Caulobacter crescentus

**Christine Jacobs-Wagner**, Yale University, New Haven, CT, USA

**9:00 – 9:30 am** Structural Biology of Cytoskeletal Proteins

**Jan Löwe, LMB**, Cambridge, UK

**9:30 – 9:50 am** The Bacterial Cytoskeleton: A New Role in Morphogenesis for the Actin-lik Protein MreBH of *Bacillus subtilis*

**Rut Carballido-López**, I.N.R.A., Jouy-en-Josas, France

**9:50 – 10:10 am** The MreB actin Homolog Mediates Both Cell Polarity and Chromosome Segregation in *Caulobacter crescentus*

**Zemer Gitai**, Princeton University, Princeton, NJ, USA

**10:10 – 10:40 am** **Coffee Break and Poster Viewing**

**10:40 – 11:10 am** *E. coli* Cell Division; FtsZ

**Joe Lutkenhaus**, University of Kansas, Kansas City, KS, USA

**11:10 – 11:30 am** Domain Architecture and Structure of the Major Extracytoplasmic Domain of the Divisomal Protein DivIB
**Glenn F. King**, University of Connecticut Health Center, Farmington, CT, USA

**11:30 – 11:50 am** Minimizing the Divisome: Compensating for the Loss of Essential Cell Division Protein FtsK in *E. coli*
**William Margolin**, University of Texas Medical School, Houston, TX, USA

**11:50 – 12:10 pm** Developmental Regulation of Chromosome Replication by Protein Binding and Proteolysis
**Gregory T. Marczynski**, McGill, Montreal, PQ, Canada

**4:00 – 6:00 pm** **Poster session #1**

**6:00 – 7:15 pm** **Dinner**

**Session 3: Morphogenesis**

Session Chair: Larry Shimkets

**7:30 – 8:00 pm** *Bacillus* Engulfment and Polarity
**Kit Pogliano**, University of California, La Jolla, CA, USA

**8:00 – 8:30 pm** Developmental Control of Surface Adhesion in *Caulobacter*
**Yves Brun**, Indiana University, Bloomington, IN, USA

**8:30 – 8:50 pm** Apical Complexes of DivIVA and Their Role in Establishing the Polarised Growth of Streptomvces Coelicolor

**Klas Flärdh**, Lund University, Lund, Sweden

**8:50– 9:10 pm** Control of Peptidoglycan Synthesis During Sporulation of *Streptomyces coelicolor*

**Gilles P. van Wezel**, Leiden University, Leiden, The Netherlands

**9:10 – 9:30pm** Linking Gene Transcription to Morphogenesis

**David S. Rudner**, Harvard Medical School, Boston, MA, USA

**9:30 - 9:50 pm** Developmental Characterization of The *Myxococcus xanthus* nla 18 mutant Reveals a Defect in ppGpp Accumulation

**Michelle E. Diodati**, UC Davis, Davis, CA, USA

## FRIDAY JULY 15TH

**7:00 – 8:15 am** **Breakfast**

**Session 4: Developmental Transcription**
Session Chair: Charlie Moran

**8:30 – 9:00 am** Cannibalism

**Rich Losick**, Harvard University, Cambridge, MA, USA

**9:00 – 9:30 am** The *Streptomyces* Developmental Proteins WhiB and WhiD Carry a [4Fe-4S] Cluster

**Mark Buttner**, John Innes Centre, Norfolk, UK

**9:30 – 10:00 am** A Systems-Level Approach to Regulation of Cell Cycle Progression and Development in *Caulobacter crescentus*

**Michael Laub**, Harvard University, Cambridge, MA, USA

**10:00 – 10:30 am** **Coffee Break and Poster Viewing**

**10:30 – 10:50 am** Serine Proteases from Two Cell Types Form a Cascade that Governs Regulated Intramembrane Proteolysis of Pro- Sigma$^{K}$ during *Bacillus subtilis* Development

**Lee Kroos**, Michigan State University, East Lansing, MI, USA

**10:50 – 11:10 am** Developmental Commitment in a Bacterium

**Jonathan Dworkin**, Columbia University, New York, NY, USA

**11:10 – 11:30 am** Coordinating Developmental Gene Expression in *Myxococcus xanthus*

**Anthony G. Garza,** Syracuse University, Syracuse, NY, USA

**11:30 – 11:50 am** Structural Proteins Involved in Differentiation of Streptomycetes

**Dennis Claessen**, University of Oxford, Oxford, UK

**11:50 – 12:10 pm** The Chaplins of *Streptomyces Coelicolor*: Important Developmental Proteins that are Associated with the Tat Secretion Pathway

**Marie Elliot**, McMaster University, Hamilton, ON, Canada

**4:00 – 6:00 pm** **Poster session #2**

**6:00 – 7:15 pm** **Dinner**

**Session 5: Developmental Diversity & Symbiosis**

Session Chair: Joanne Willey

**7:30 – 8:00 pm** Development of *Rhizobium leguminosarum* bacteroids in pea nodules

**Phil Poole**, University of Reading, Reading, UK

**8:00 – 8:30 pm** Sequential Bacterial-host Signaling Leads to Light Organ Development and Symbiosis

**Edward Ruby**, University of Wisconsin, Madison, WI, USA

**8:30 – 9:00 pm** The Cyanobacterial Circadian Clock

**Susan Golden**, Texas A & M University, College Station, TX, USA

**9:00 – 9:20 pm** Predatory *Bdellovibrio*: Gene Expression from Attack Phase to the Bdelloplast

**Liz Sockett**, University of Nottingham, Nottingham, UK

**9:20 – 9:40 pm** Identification and Characterization of Five Small RNAs that Control Quorum Sensing in *Vibrio harveyi*

**Kim Tu**, Princeton University, Princeton, NJ, USA

## SATURDAY JULY 16TH

**7:00 – 8:15 am** **Breakfast**

**Session 6: Signaling**
Session Chair: Austin Newton

**8:30 – 9:00 am** Signaling the Regulatory Network for *Myxococcus xanthus* Fruiting Body Development
**Dale Kaiser**, Stanford University, Stanford, CA, USA

**9:00 – 9:30 am** Signaling in Heterocyst Differentiation
**Jim Golden**, Texas A & M University, College Station, TX, USA

**9:30 – 10:00 am** Spatial Regulation of a Signaling Pathway
**Judy Armitage**, Oxford University, Oxford, UK

**10:00 – 10:30 am** **Coffee Break**

**10:30 – 10:50 am** Sensing DNA Damage Upon Entry into Sporulation in *Bacillus subtilis*
**Sigal Ben-Yehuda**, The Hebrew University of Jerusalem, Jerusalem, Israel

**10:50 – 11:10 am** Biochemical and Genetic Identification of a c-di-GMP Binding Motif
**Beat Christen**, University of Basel, Basel, Switzerland

**11:10 – 11:30am** Sensing Signals through the Frz Signal Transduction System of *Myxococcus xanthus*
**David R. Zusman**, University of California, Berkeley, CA, USA

**11:30 – 11:50 am** The Dif Chemosensory Pathway is Directly Involved in Phosphatidylethanolamine Sensory Transduction in *Myxococcus xanthus*

**Pamela J. Bonner**, University of Georgia, Athens, GA, USA

**11:50 – 12:10 pm** Analysis of Signal Transduction and Chemotaxis in *Myxococcus xanthus*

**John R. Kirby**, Georgia Institute of Technology, Atlanta, GA, USA

**Session 7: Multicellularity**
Session Chair: Patricia Hartzell

**2:00 – 2:30 pm** Investigating *Streptomyces* Developmental Biology in the Genomic Era

**Keith Chater**, John Innes Centre, Norfolk, UK

**2:30 – 3:00 pm** Rodk, an Unusual Hybrid Histidine Protein Kinase, Regulates Multiple Steps during Fruiting Body Formation in *Myxococcus xanthus*

**Lotte Sogaard-Andersen**, Max Planck Institute, Marburg, Germany

**3:00 – 3:30 pm** Developmental Biology of Biofilms

**Roberto Kolter,** Harvard University, Cambridge, MA, USA

**3:30 - 3:50 pm** Metabolome Analyses of *Myxococcus xanthus* Reveal Biomarkers of Myxobacterial Development

**Helge B. Bode,** Saarland University, Saarbrücken, Germany

**3:50 – 4:10 pm** Inhibition of Biofilm Formation by *Bacillus subtilis* Through High Levels of Spo0A~P

**Beth A. Lazazzera,** UCLA, Los Angeles, CA, USA

**4:10 – 4:30 pm** Purification and Structural Determination of SapT, a Lantibiotic-Like Peptide Involved in Aerial Hyphae Formation in the Streptomycetes

**Joanne M. Willey**, Hofstra University, Hempstead, NY, USA

**6:00 – 10:00 pm** **Banquet**

## S1:2

### SLMA, A NUCLEOID-ASSOCIATED PROTEIN THAT COORDINATES CYTOKINESIS WITH CHROMOSOME POSITIONING IN *E.COLI*

***Piet de Boer,*** *Thomas Bernhardt*

*Dept. of Molecular Biology & Microbiology, Case School of Medicine, Cleveland, Ohio*

Cytokinesis in most prokaryotes starts with assembly of the tubulin-like FtsZ protein into a ring just underneath the cell membrane. Spatial control of ring assembly in *E.coli* is achieved by the Min system and by nucleoid occlusion (NO), two partially redundant inhibitory systems that prevent formation of the Z ring at off-center sites. The Min system is intensely studied, and is proposed to force FtsZ assembly to midcell by the rapid pole-to-pole oscillation of the FtsZ polymerization antagonist MinC. In contrast, little is known about NO, which couples positioning of the division plane to that of the chromosomes by somehow blocking Z ring assembly in the close vicinity of nucleoids.

Although Min$^-$ cells are viable, we reasoned that Min might become essential to cells that are also impaired in NO. We developed a generally applicable synthetic lethal screen for *E. coli* and used it to isolate mutations synthetically lethal with a defective Min system (*slm* mutants). Two such mutations mapped to an ORF (*slmA*), encoding a protein with many features expected of a NO factor. i) SlmA is a DNA-binding protein, and functional fusions to GFP localize to the nucleoid. ii) Neither depletion nor overproduction of SlmA affect cellular FtsZ levels. iii) Yet, SlmA$^-$ Min$^-$ cells contain numerous FtsZ assemblies over nucleoids, and fail to divide unless FtsZ is overproduced. iv) In contrast to SlmA$^+$ cells, SlmA$^-$ cells form septa over the centrally located nucleoid of cells in which initiation of DNA replication is blocked. Thus, SlmA is required for the ill-defined antiguillotine checkpoint that normally prevents chromosome 'cutting' under these conditions. v) Overproduction of SlmA inhibits cell division by preventing Z ring assembly. Interestingly, rather than forming membrane-associated rings, FtsZ co-localizes with SlmA on the nucleoids of such cells. These and additional results suggest that nucleoid-bound SlmA promotes NO by modulating FtsZ polymerization directly.

Although SlmA shares many physiological properties with a recently discovered NO factor in *B.subtilis* (Noc), the proteins appear unrelated.

## S1:3

### POSITIONAL CONTROL OF REPLICATION IN *BACILLUS SUBTILIS*

***M. B. Berkmen****, A. D. Grossman;*
*Massachusetts Institute of Technology, Cambridge, MA*

In *Bacillus subtilis* and other bacteria, both the origin of replication and the replication machinery (replisome) occupy characteristic subcellular positions. The mechanisms by which origins and replisomes reach their characteristic positions have yet to be determined, but since the two must interact during initiation of replication, it is possible that the localization of one influences or determines the position of the other. In order to gain additional insight into the *B. subtilis* replication cycle, we simultaneously visualized replication origins and the replication machinery inside live cells. Three observations led us to favor the model that the position of the replisome is established by the location of the origin of replication at the time of initiation. First, origin regions are properly positioned prior to replisome focus appearance at a given site. Second, replisomes appear at sites only once a replication origin has been previously positioned there. Third, replisomes are aberrantly positioned inside cells where the origin of replication has been artificially mislocalized. Origins are located at or near midcell both prior to and after replisome focus appearance, providing further support that replication initiates near midcell. In addition, time-lapse microscopy revealed that single replisome foci reversibly split into two closely spaced foci in a subset of cells, including cells that contain a single origin focus. This result indicates that sister replication forks may not be intimately associated with each other during the replication cycle. Taken together, our results are consistent with the emerging picture that the replisome is at a characteristic subcellular location but that it is not locked in place. Based on our results combined with previous work, we have developed a more detailed model of the spatial and temporal organization of the *B. subtilis* replication cycle.

## S1:4

### COORDINATION OF CHROMOSOME PARTITIONING AND CYTOKINESIS IN CAULOBACTER

***J. W. Gober****, J. Easter;*
*UCLA, Los Angeles, CA*

In *Caulobacter crescentus* the partitioning proteins ParA and ParB operate a molecular switch that couples chromosome partitioning to cytokinesis. ParB has been shown to bind to sequences adjacent to the origin of replication (*parS*) and localize to the poles of the predivisional cell. Depletion of

ParB results in a severe cell division defect with the formation of filamentous cells that lack the ability to form FtsZ rings, the earliest step in cytokinesis. ParA is an ATPase that can stably exist in an ADP- or ATP-bound state. The two different nucleotide-bound forms of ParA differ in their ability to bind single-stranded DNA and influence ParB-*parS* interaction. ParB regulates this switch by promoting nucleotide exchange. Overexpression of ParA resulted in a cell division phenotype that was identical to ParB-depleted cells, whereas overexpression of both proteins resulted in a partitioning defect, but no evident cell division defect. Overexpression of ParA and depletion of ParB both led to an increase in the ratio of ParA/ParB in the cell and, more importantly, a significant increase in the fraction of ParA that was bound to ADP. These observations suggest that that ParA-ADP functions as a cell division inhibitor: the formation of two partitioning complexes (ParB bound to *parS*) at the cell poles stimulates exchange of ADP for ATP and thus relieves the cell division block. Using in vitro assays we have found that purified ParA can directly influence the polymerization of FtsZ. ParA-ADP inhibited polymerization induced by the addition of $Mg^{++}$ to purified *Caulobacter* FtsZ, whereas ParA-ATP appeared to increase the rate of FtsZ polymerization. We have also found that ParB can regulate the formation of FtsZ polymers by stimulating ParA nucleotide exchange. These results suggest that ParB-regulated nucleotide exchange may act as a positive signal in promoting FtsZ ring formation in vivo. Interestingly, we have found that transient overexpression of ParA resulted in the relatively rapid appearance of FtsZ foci that were restricted to the cell poles. We hypothesize that the presence of ParB, which is also present at the cell poles under these conditions, stimulates FtsZ polymerization by maintaining polarly-localized ParA in the ATP-bound state. Does ParA-ATP influence formation of FtsZ rings at their normal midcell location? This would require that ParB localize to the midcell in order to promote the formation of ATP bound ParA. In order to test this idea, we assayed the localization of ParB and found that a small fraction of stalked cells contained ParB foci that were localized to one pole and at the midcell. Cells possessing this pattern of localization never had an additional focus of ParB at the other cell pole, suggesting that the appearance of ParB at the midcell represents a transient DNA partitioning intermediate that may also serve to stimulate FtsZ ring formation via promoting ParA nucleotide exchange.

# S1:5

## MULTIPLE INTERACTIONS BETWEEN THE TRANSMEMBRANE DIVISION PROTEINS AND CELL ELONGATION PROTEINS OF *B. SUBTILIS*

***R. Daniel;***
*Univ. of Oxford, Oxford, UNITED KINGDOM*

Despite many decades of research, cell division remains an important unsolved problem in bacteria. About 10 conserved proteins required for cell division have been identified through the isolation of conditional mutations resulting in a filamentous phenotype. The application of microscopic techniques has shown that these proteins assemble at the site of division prior to constriction, and has allowed a dependence pattern for assembly to be worked out for *E. coli* and *B. subtilis*. We have been focussing on the functions and assembly of the essential transmembrane components of the division apparatus (PbpB, FtsL, DivIC, DivIB and FtsW) in *B. subtilis*. Interactions between these proteins have been analysed by a range of methods, including genetic suppression, yeast and bacterial 2-hybrid screens, and biochemistry. The results have revealed a complex web of interactions, in which, remarkably, almost all of the proteins interact with themselves and with most of the other components. The key conclusions to date are as follows. 1. The N-terminal non-catalytic domain of PBP 2B (= FtsI) is the site of interactions with the other division proteins and probably plays a key role in assembly of the machinery. 2. FtsL and DivIC form a heterodimeric/polymeric complex that interacts with the other proteins. 3. DivIB controls the turnover of FtsL and DivIC, which may be involved in the disassembly of the division machinery during division. 4. Components of the cell elongation machinery also interact with and co-localize with the division machinery, suggesting that both wall synthetic machines operate at the division site in this organism.

# S2:1

## CYTOSKELETON AND CELL SHAPE IN CAULOBACTER CRESCENTUS

***C. Jacobs-Wagner;***
*Yale University, New Haven, CT*

Similarly to eukaryotic cells, prokaryotic cells come in a variety of shapes. Despite the apparent simplicity of bacteria and their amenability to genetic and biochemical experimentation, little is known about the molecular basis of bacterial cell shape determination and maintenance. In eukaryotes, the cytoskeleton, which is made of microtubules, microfilaments and intermediate filaments, constitutes an internal framework that is essential for the maintenance of cell shape. It is now apparent that bacteria also possess an actin-like cytoskeleton made of MreB and that this cytoskeleton is involved in determining rod cell morphology. Our laboratory has recently discovered a prokaryotic counterpart of intermediate filament (IF) proteins, termed crescentin. Crescentin is a fibrous protein of 430 residues with a tripartite domain architecture similar to that of eukaryotic IF proteins. Purified crescentin self-assembles spontaneously into ~10 nm wide filaments in vitro without exogenous energy sources or accessory cofactor requirements, which is a distinct biochemical property of IF proteins. The function of crescentin is required for the characteristic crescent shape of Caulobacter crescentus as cells lacking crescentin lose their curvature and adopt a straight-rod cell morphology. Consistent with its role in cell curvature, crescentin forms a filamentous structure along the inner cell curvature of wild-type cells. Co-immunoprecipitation experiments have identified several proteins that may interact

with crescentin. The role of these proteins in cell curvature will be discussed.

## S2:3

### THE BACTERIAL CYTOSKELETON: A NEW ROLE IN MORPHOGENESIS FOR THE ACTIN-LIKE PROTEIN MREBH OF *BACILLUS SUBTILIS*

***R. Carballido López***[1]*, D. Ehrlich*[1]*, J. Errington*[2]*, P. Noirot*[1]*;*
*[1]I.N.R.A., Jouy-en-josas, FRANCE, [2]Sir William Dunn School of Pathology, University of Oxford, UNITED KINGDOM*

**Background**: Bacterial MreB proteins have recently been identified as actin orthologues. The rod-shaped *B. subtilis* has 3 isoforms: MreB, Mbl and MreBH, which assemble into similar but distinct helical structures around the periphery of the cell. MreB and Mbl (MreB-like) are involved in shape determination by controlling functions related to the bacterial envelope. Mbl is required for lateral cell wall (CW) expansion by directing the insertion of peptidoglycan in a helical pattern. MreB has a role in the control of cell width. The mechanism to achieve this is still unclear but MreB might influence the synthesis, structure or organization of the CW. MreB has also been implicated in chromosome segregation. However, virtually nothing was know about the function of MreBH. **Results:** We have characterised a role of MreBH in morphogenesis. The phenotype of *mreBH* mutants and the subcellular localization of a GFP-MreBH fusion were analysed. A genome-wide yeast two-hybrid screen was used to identify proteins of *B. subtilis* that physically associate with MreBH. MreBH was shown to interact specifically with LytE, a CW hydrolase. *LytE* mutants displayed a phenotype similar to that of *mreBH* mutants. A functional LytE-GFP fusion was produced and processed. The signal peptide of LytE was shown to be cleaved off. LytE-GFP localized predominantly to the cell separation sites, but also along the cylinder in a pattern suggestive of a helical distribution. Lateral wall localization was dependent on MreBH (elongation). Septal localization was dependent on FtsZ (division). The topological problem raised by the fluorescence of a GFP fusion presumed to be secreted was also addressed. Strikingly, both the LytE-GFP fusion protein and the GFP fluorescence were shown to be in the extracellular CW fraction. **Conclusions**: MreB-like proteins are membrane-associated spatial regulators of CW biogenesis in *B. subtilis*. Whilst Mbl directs lateral CW insertion, MreBH controls lateral CW hydrolysis. By targeting the autolysin LytE to specific sites of action, MreBH might direct distribution of new material, release of torsional stress and probably turnover of the CW in a coordinated manner that allows the rod-shaped cell to elongate whilst keeping its diameter constant. No link between cell growth and autolysins has been clearly shown before. Furthermore, the MreBH-LytE interaction reveals how the inside-to-outside cross-talk is mediated. Another striking finding reported here is the localization of a secreted protein in a Gram+ bacterium using a GFP fusion. The mechanism and the transport system allowing the GFP to be exported in a folded conformation are under study.

## S2:4

### THE MREB ACTIN HOMOLOG MEDIATES BOTH CELL POLARITY AND CHROMOSOME SEGREGATION IN *CAULOBACTER CRESCENTUS*

***Z. Gitai***[1]*, L. Shapiro*[2]*;*
*[1]Princeton Univ., Princeton, NJ, [2]Stanford Univ., Stanford, CA*

Bacteria, like all cells, have complex subcellular architectures with macromolecular complexes that are localized to specific places at specific times in the cell cycle. The mechanisms by which cells achieve their subcellular organization are fundamental to understanding how they grow, divide, differentiate and communicate. To study these processes, we focus on the bacterium *Caulobacter crescentus,* whose unique life cycle culminates in an asymmetric cell division to produce daughters with different morphologies and fates. Our exploration of *Caulobacter* cell biology has centered on the bacterial cytoskeleton, with an emphasis on the actin homolog, MreB. During the *Caulobacter* cell cycle, MreB undergoes dynamic rearrangement involving a spiral that collapses into a ring in a slinky-like fashion. We find that this dynamic MreB structure is essential for viability and regulates cell morphogenesis, polarity, and chromosome segregation, thereby filling multiple voids in our understanding of *Caulobacter* biology. By conditionally depleting and re-expressing MreB we demonstrated that MreB dictates the proper polar destinations of cell-cycle regulatory proteins. MreB thus plays an instructive role in determining polar protein localization. Meanwhile, we have employed temporal studies with an MreB-inhibiting drug and MreB-ChIPs to identify specific chromosomal loci that direct chromosome movement by associating with MreB. These loci appear to function as a bacterial centromere. Our findings suggest a two-step model for *Caulobacter* chromosome segregation: the origin-proximal region is first segregated by an MreB-dependent mechanism, followed by MreB-independent segregation of the rest of the chromosome. Together, our results point to a key role for MreB in integrating global positional information. To understand the mechanisms whereby MreB executes these cellular functions, we are implementing new methodologies for ultra-high-resolution visualization of MreB ultrastructure, and using single-molecule imaging to examine the polarity and dynamics of MreB polymers.

# S2:6

## DOMAIN ARCHITECTURE AND STRUCTURE OF THE MAJOR EXTRACYTOPLASMIC DOMAIN OF THE DIVISOMAL PROTEIN DIVIB

*S. A. Robson,* ***G. F. King****;*
*University of Connecticut Health Center, Farmington, CT*

Bacterial cytokinesis requires the coordinated assembly of a complex of essential cell division proteins, collectively known as the divisome, at the incipient division site. DivIB/FtsQ is a conserved component of the divisome in bacteria with cell walls, suggesting it plays a role in synthesis and/or remodeling of septal peptidoglycan. We demonstrate that the extracytoplasmic region of DivIB comprises three structurally discrete domains, designated α, β, and γ from N- to C-terminus. The γ domain, which is essential for DivIB function, is unstructured in the absence of other divisomal proteins. The β domain comprises the bulk of the extracytoplasmic region of DivIB and its structure is unique, with conserved surface features that likely mediate interactions with other divisomal proteins. The α domain is proximal to the cytoplasmic membrane and coincident with the previously predicted polypeptide-transport-associated (POTRA) domain. The α domain might serve as chaperone for FtsL, which forms a ternary complex with DivIB and DivIC, the unstable γ domain, or some other divisomal component. This work provides the first structural insights into DivIB/FtsQ and should facilitate elucidation of its role(s) in cell division.

# S2:7

## MINIMIZING THE DIVISOME: COMPENSATING FOR THE LOSS OF ESSENTIAL CELL DIVISION PROTEIN FTSK IN E. COLI

*B. Geissler,* ***W. Margolin****;*
*University of Texas Medical School, Houston, TX*

In Escherichia coli, at least twelve proteins colocalize to the cell midpoint, assembling into a membrane-associated protein machine that forms the division septum. Inactivating any of these proteins blocks cell division and causes cell filamentation. Many of these proteins, including FtsK, are essential for viability but their functions in cell division are unknown. We have now determined that the essential function of the membrane domain of FtsK in cell division can be partially bypassed. Cells containing either the ftsAR286W mutation or a plasmid carrying the ftsQAZ genes suppressed a ftsK44(ts) allele efficiently. Moreover, ftsAR286W or multicopy ftsQAZ, either of which could suppress the requirement for the essential cell division gene zipA, allowed cells with a complete deletion of ftsK to survive and divide, although many of these ftsK null cells formed multiseptate chains. FtsQ, FtsI, and FtsN, which normally depend on FtsK to localize to division sites, were able to localize to division sites in the absence of FtsK, indicating that FtsK is not directly involved in their recruitment. Cells expressing additional ftsQ, and to a lesser extent ftsB and ftsN, were able to survive and divide in the absence of ftsK, although cell chains were often formed. Surprisingly, the cytoplasmic and transmembrane domains of FtsQ, while not sufficient to complement an ftsQ null mutant, were sufficient to confer viability and septum formation in the absence of ftsK. These findings suggest that the membrane domain of FtsK is normally involved in stability of the division protein machine and exhibits functional overlap with FtsQ, FtsB, and FtsN.

# S2:8

## DEVELOPMENTAL REGULATION OF CHROMOSOME REPLICATION BY PROTEIN BINDING AND PROTEOLYSIS

***G. T. Marczynski****;*
*McGill, Montreal, PQ, CANADA*

Most bacteria apparently use the DnaA protein to bind their replication origins and to initiate chromosome replication. Along with the ubiquitous DnaA, the dimorphic bacterium *Caulobacter crescentus* also uses response-regulator CtrA to regulate chromosome replication. The evolutionary recruitment of CtrA may reflect a special need for a more precise coordination between development and replication. While CtrA has 5 conspicuous high affinity binding sites inside the *C. crescentus* replication origin (*Cori*), the binding sites for DnaA have remained elusive. Using *in vitro* foot-printing with pure *C. cresentus* DnaA protein, we have now identified two primary and several secondary DnaA binding sites. Interestingly both primary DnaA binding sites overlap with CtrA binding sites, implying direct contacts between DnaA and CtrA during replication control. Cell development is coordinated with proteolysis and previous studies demonstrated that CtrA proteolysis by ClpXP helps restrict chromosome replication to the dividing cell-type. We observe that *C. crescentus* DnaA protein is also selectively targeted for proteolysis. However, DnaA proteolysis uses a different mechanism. DnaA protein is unstable during both growth and stationary phases. During growth phase, DnaA proteolysis ensures that primarily newly made DnaA protein initiates chromosome replication during each cell-cycle. Upon entry into stationary phase, DnaA protein is completely removed while CtrA protein is retained. Cell-cycle arrest by sudden carbon or nitrogen starvation is sufficient to increase DnaA proteolysis, and relieving starvation rapidly stabilizes DnaA protein. This starvation-induced proteolysis completely removes DnaA protein even while DnaA synthesis continues. This very surprising result implies that *C. crescentus* primarily relies on proteolysis, and not transcription, to adjust DnaA in response to rapid nutritional changes. The *C. crescentus* ClpP

protease selectively degrades DnaA. However a dominant-negative *clpX* allele, that blocks CtrA degradation, even when combined with a *clpA* null allele, did not decrease DnaA degradation. We suggest that either a novel chaperone presents DnaA to ClpP or that ClpX is used with exceptional efficiency so that when ClpX activity is limiting for CtrA degradation it is not limiting for DnaA degradation. This unexpected and finely tuned proteolysis system may be an important adaptation for a developmental bacterium that is often challenged by nutrient-poor environments.

## S3:2

### DEVELOPMENTAL CONTROL OF SURFACE ADHESION IN *CAULOBACTER*

***Yves Brun***,. *Wang, K. Flärdh;*
*Indiana University, Bloomington, IN*

Single cells of *Caulobacter crescentus* attach to surfaces with one of the strongest biological adhesins, the polar holdfast. However, holdfasts are not sufficient for efficient adhesion. Adhesion occurs as a result of a developmentally controlled multistep process. Initial surface binding occurs in the swarmer stage of the cell cycle and is mediated by flagellar motility and pili. Holdfast synthesis at the pole containing the flagellum and pili cements the cell to the surface during swarmer to stalked cell differentiation. Stalk synthesis at the same pole results in a cell attached to the surface through the holdfast at the tip of the stalk. Coordination of the various stages leading to adhesion is mediated by the polar localization factor, PodJ, and the histidine kinase, PleC. Synthesis of the holdfast requires a set of polysaccharide synthesis, export, and attachment proteins, many of which are localized to the appropriate cell pole in a cell cycle dependent fashion.

## S3:3

### APICAL COMPLEXES OF DIVIVA AND THEIR ROLE IN ESTABLISHING THE POLARISED GROWTH OF STREPTOMYCES COELICOLOR

*S. Wang,* ***K. Flärdh****;*
*Lund University, Lund, SWEDEN*

In contrast to the situation in most other bacteria, the Streptomyces cell wall is polymerised at one cell pole, the hyphal tip. This pronounced tip extension is analogous to apical growth of filamentous fungi. It is not clear how the bacterial cell wall synthesis machinery is recruited to and organised at the hyphal tips, and how cell shape is determined at these sites in streptomycetes. Interestingly, the cell poles (hyphal tips) at which apical growth occurs are not generated by cell division, and each hyphal tip is instead established de novo as a lateral branch or germ tube in a process that is independent of septation and FtsZ. We have previously shown that the S. coelicolor homologue of DivIVA has an important role in hyphal tip growth. It localises distinctively to hyphal tips and nascent lateral branches. DivIVA was essential for growth, and partial depletion produced a phenotype strikingly similar to tip growth or nuclear migration mutants in fungi. Also, overexpression had dramatic effects on shape determination, leading to conspicuous swollen and pear-shaped cells. We can now show that induction of divIVA overexpression in pre-formed hyphae, that already contained a the DivIVA-EGFP hybrid protein at hyphal tips, rapidly lead to reorganisation of the apical DivIVA assemblage and to formation of several new discrete foci of DivIVA along the lateral walls. At those new foci, apical growth was initiated and branch-like lateral outgrowths emerged. Thus, DivIVA not only targets tips and affects morphogenesis, but is also instrumental in establishment of new tips. Furthermore, we report that DivIVA oligomerises and forms large protein complexes both in vitro and in vivo. The nature, composition, and shape of these complexes have been investigated with several methods. Based on these observations, it can be suggested that DivIVA forms large apical protein complexes in Streptomyces that are reminiscent of a cytoskeletal element, and have an important role in re-orienting cell polarity upon branching and in establishing tip extension, presumably by recruiting the machinery for cell wall assembly to the hyphal tip.

## S3:4

### CONTROL OF PEPTIDOGLYCAN SYNTHESIS DURING SPORULATION OF STREPTOMYCES COELICOLOR

***G. P. van Wezel***[1]*, E. E. Noens*[1]*, H. K. Koerten*[2]*;*
*[1]Leiden University, Leiden, THE NETHERLANDS, [2]Leiden University Medical Centre, Leiden, THE NETHERLANDS*

Developmental cell division in sporulation-committed aerial hyphae of streptomycetes involves the synchronous generation of up to a hundred septa, in close harmony with chromosome segregation. This intriguing process requires a complex coordination of cell wall synthesis, DNA segregation, and autolysis, and while *Streptomyces* development is widely studied, our knowledge of the mechanisms that direct the synthesis and autolysis of the spore walls is based primarily on parallels to other bacterial systems. Here we will present an overview of our recent results concerning proteins that play an important role in the synthesis and stability of the spore-wall peptidoglycan during the later stages of the sporulation process. The SsgA-like proteins (SALPs) are unique to sporulating actinomycetes, and each of the seven members of *S. coelicolor* plays its own specific role in spore morphogenesis, most likely through direct interaction with the enzymes responsible for build up (penicillin binding proteins or PBPs) and degradation (autolysins) of the spore peptidoglycan. The actin-like cytoskeletal protein MreB plays a crucial role in providing stability to the aerial hyphae and spores, and deletion of either genes of the *mreBCD* operon severely compromises

the resilience of the spores and hyphae, although septum formation is not affected. Finally, we showed, in collaboration with the groups of Titgemeyer (Erlangen) and Rigali (Liege), that the pleiotropic-acting transcription factor DasR primarily controls the fate of N-acetylglucosamine, one of the major components of the peptidoglycan. A *dasR* mutant has defective spores, due to severe detachment of the cytoplasmic membrane from the spore-wall peptidoglycan. A new model of spore wall synthesis and spore maturation will be presented, integrating our most recent findings on the functions of these three classes of genes.

# S3:5

## LINKING GENE TRANSCRIPTION TO MORPHOGENESIS

*T. Doan, K. Marquis,* ***D. Z. Rudner****;*
*Harvard Medical School, Boston, MA*

Through out the process of sporulation the forespore and mother cell follow completely different programs of gene expression but these programs are linked through signal transduction pathways. $\sigma^K$ directs late gene expression in the mother-cell compartment and its activity is tightly regulated to ensure that transcription of these genes is held in register with events occurring in the forespore. $\sigma^K$ is synthesized as an inactive precursor protein (pro-$\sigma^K$) and is activated by proteolytic processing. The conversion of pro-$\sigma^K$ to $\sigma^K$ requires the mother cell membrane protein SpoIVFB, which is likely to be the processing enzyme. SpoIVFB is held inactive by two other integral membrane proteins, SpoIVFA and BofA. All three proteins reside in a multimeric membrane complex that localizes with exquisite specificity to the mother-cell membrane that surrounds the forespore (the outer forespore membrane). The processing of pro-$\sigma^K$ is triggered by a signal protein, SpoIVB, which is produced in the forespore under the control of $\sigma^G$ and secreted into the space between the mother-cell and forespore membranes where it overcomes SpoIVFA and BofA inhibition of SpoIVFB. We have recently identified four mother cell proteins (SpoIID, SpoIIP, SpoIIM, SpoIIIAH) and one forespore protein (SpoIIQ) that are all required to efficiently anchor the pro-$\sigma^K$ signaling complex in the outer forespore membrane. We have investigated whether these five proteins play a *direct* role in the pro-$\sigma^K$ processing pathway or only participate indirectly by anchoring the signaling complex. All five anchoring proteins are required at earlier stages in the sporulation pathway (IID, IIP, IIM, and IIQ are required for engulfment and all five proteins are required for $\sigma^G$ activation). Thus, in order to investigate the role of these proteins in the $\sigma^K$ signaling pathway, we used a collection of $\sigma^G$ bypass mutants. Suprisingly, we have discovered that all five proteins are required for efficient pro-$\sigma^K$ processing. Interestingly, these proteins appear to act at different steps in the signaling pathway. These results suggest that $\sigma^K$ activation is coupled to both the activation of $\sigma^G$ and the morphological process of engulfment.

# S3:6

## DEVELOPMENTAL CHARACTERIZATION OF THE MYXOCOCCUS XANTHUS NLA18 MUTANT REVEALS A DEFECT IN PPGPP ACCUMULATION

***M. E. Diodati***[1]*, F. Ossa*[2]*, N. B. Caberoy*[2]*, I. R. Jose*[1]*, A. G. Garza*[2]*, M. H. Singer*[1]*;*
[1]*UC Davis, Davis, CA,* [2]*Syracuse University, Syracuse, NY*

When *Myxococcus xanthus* cells accumulate the intracellular starvation signal (p)ppGpp during amino acid starvation, they initiate a multicellular developmental process that yields spore-filled fruiting bodies. We are interested in understanding the pathway between the accumulation of (p)ppGpp and the expression of the earliest developmental genes, and identifying key players involved in the cell's decision to undergo the vegetative growth to development transition in response to starvation.
The earliest of the (p)ppGpp-dependent genes share a common regulatory feature, a $\sigma^{54}$-like promoter. These types of promoters require positive activation by an NtrC type of activator protein.
Characterization of insertion mutants in 28 putative *ntrC*-like activator genes (*nla* genes) and analysis of their developmental phenotypes, identified an *nla* mutant with strong vegetative and developmental defects (Caberoy, et al. 2003). Designated *nla18*, this mutant had a generation time of 10.5-16 hours compared to 5 hours for wild type cells, and exhibited a delayed and incomplete developmental phenotype.
Based on both β-galactosidase assays and RNA slot blot analyses, the *nla18* mutants are defective in the expression of early developmental *Tn5lac* transcriptional fusions, including *sdeK* (Ω4408) and *spi* (Ω4521). In addition, *nla18* cells have a partial defect for the production of the relA-dependent population density signal, A-signal. Using ppGpp accumulation assays with isogenic wildtype and *nla18* cells, we show that the levels of ppGpp in *nla18* cells are about 50% of wild type during vegetative growth and about 18% of wild type under starvation conditions. We propose that, the relatively low levels of ppGpp in the *nla18* mutant lead to the observed defects in fruiting body formation, cell-cell signal production, and developmental gene expression. Currently, vegetative microarray analyses are underway and may yield clues to Nla18's specific targets and its inability to initiate an appropriate starvation response.

# S4:1

## CANNIBALISM

***R. Losick;***
*Harvard University, Cambridge, MA*

Certain bacteria have evolved an elaborate response to conditions of nutrient limitation in which they metamorphose into a resting cell or spore that can resist extremes of environment and time. But what if nutrient limitation is fleeting? Bacteria that willy-nilly launch into the multi-hour process of spore formation in response to fluctuating nutrient availability might be at a competitive disadvantage relative to non-spore forming bacteria. On the other hand, dallying in response to a famine might deplete energy reserves needed for successful morphogenesis. In other words, the decision to sporulate is not to be taken lightly!
The spore-forming bacterium *Bacillus subtilis* optimizes its chances of getting the decision right by a strategy of commitment and cannibalism. A hallmark of sporulation is the formation of a polar division septum. Up until the time the polar septum is formed sporulation is reversible, and the replenishment of nutrients allows the cell to return to a state of growth and binary fission. Once polar division has taken place, however, the cell is committed to completing morphogenesis whether or not nutrients re-appear.
*B. subtilis* uses cannibalism to prolong its period of pre-divisional uncertainty for as long as possible. Cells that have entered the pathway to sporulate but have not yet produced a polar septum export protein factors that kill non-sporulating, sibling cells. The sporulating bacteria then feed on the nutrients released from the dead siblings. This delays the time of polar division and allows the bacteria to postpone committing to morphogenesis. For this spore-forming bacterium, no price is too high, even fratricide, to optimize decision making in an uncertain world.
A focus of the presentation will be the elucidation of a novel signal transduction pathway by which an exported toxin protein induces the synthesis of an immunity protein that protects sporulating cells but not non-sporulating siblings from the action of the toxin.

# S4:2

## THE *STREPTOMYCES* DEVELOPMENTAL PROTEINS WHIB AND WHID CARRY A [4FE-4S] CLUSTER

*P. Jakimowicz[1], M. R. Cheesman[2], W. R. Bishai[3], K. F. Chater[1], A. J. Thomson[2],* ***M. J. Buttner[1];***
*[1]John Innes Centre, Norwich, UNITED KINGDOM, [2]University of East Anglia, Norwich, UNITED KINGDOM, [3]Johns Hopkins School of Medicine, Baltimore, MD*

In order to disperse themselves, streptomycetes develop specialised aerial hyphae that differentiate into chains of exospores, a process that begins with the synchronous deposition of 50 or more sporulation septa at ~1μm intervals at the tips of the hyphae. Sporulation-deficient mutants of *S. coelicolor* can be identified by virtue of their inability to synthesise the grey spore pigment, thereby remaining white, even on prolonged incubation on plates. Through this route, 14 *whi* (white) genes have been identified that are required for the production of mature spores. Two of these genes encode homologous proteins believed to function as transcription factors: WhiB, required for the initiation of sporulation septation, and WhiD, required for spore maturation. The WhiB-like (Wbl) family of putative transcription factors are present throughout the actinomycetes, but are absent from other organisms, and genetic screens have shown that members of this family play diverse, important roles in actinomycete biology. The Wbl family of proteins has four near-invariant cysteines, and we found that each of these four residues was essential for WhiD function *in vivo*, suggesting that they might act as ligands for a metal cofactor. To address this question, we over-expressed WhiD and found it contained sub-stoichiometric amounts of iron and had an absorption spectrum characteristic of a [2Fe-2S] cluster. After Fe-S cluster reconstitution under anaerobic conditions, WhiD contained a [4Fe-4S] cluster. Reconstituted WhiD gave no EPR signal as prepared, but, after reduction with dithionite, gave an EPR signal (g ~ 2.06, 1.94), consistent with a one electron reduction of a $[4Fe\text{-}4S]^{2+}$ cluster to a $[4Fe\text{-}4S]^{1+}$ state with electron spin of S=½. The anaerobically reconstituted [4Fe-4S] cluster was oxygen sensitive; exposure to air induced a change from a [4Fe-4S] to a [2Fe-2S] cluster, followed by complete loss of cluster from the protein. Essentially identical results were obtained with WhiB. These experiments raise the possibility that *Streptomyces* sporulation is redox-regulated.

# S4:3

## A SYSTEMS-LEVEL APPROACH TO REGULATION OF CELL CYCLE PROGRESSION AND DEVELOPMENT IN *CAULOBACTER CRESCENTUS*

***Michael T. Laub***
*Bauer Center for Genomics Research, Harvard University, Cambridge, MA*

Progression through the cell cycle requires the precise coordination of DNA replication, chromosome segregation, cell division, and cell growth. Regulation of these processes can be studied in the experimentally tractable bacterium *Caulobacter crescentus* where cell cycle progression includes an obligate differentiation step and the establishment of cellular polarity. Cell cycle progression in *Caulobacter* culminates in the production of two asymmetric daughter cells, a sessile stalked cell that immediately initiates a round of DNA replication and a motile swarmer cell that must first differentiate into a stalked cell before initiating DNA replication.

We have undertaken a systematic analysis of the signaling and regulatory genes controlling the *Caulobacter* life cycle, focusing primarily on the two-component signal transduction systems. We systematically generated deletion strains for each of the 106 two-component genes (62 histidine kinases and 44 response regulators) encoded in the *Caulobacter* genome. Phenotypic characterization of these mutants has identified four new two-component genes essential for viability and 16 previously uncharacterized genes required for proper cell cycle progression. As the majority of two-component genes are orphans, we also developed a systematic biochemical method, termed kinase-substrate profiling, which allows the rapid and accurate delineation of histidine kinase-response regulator cognate pairs.

Examples will be presented to demonstrate how the combination of these systematic genetic and biochemical approaches has led to the comprehensive identification of signaling pathways controlling key cell cycle events. The systematic approach has unveiled surprising connections between pathways and suggests that complex, highly branched two-component signaling systems control this organism's cell cycle.

# S4:4

## SERINE PROTEASES FROM TWO CELL TYPES FORM A CASCADE THAT GOVERNS REGULATED INTRAMEMBRANE PROTEOLYSIS OF PRO-SIGMA$^K$ DURING *BACILLUS SUBTILIS* DEVELOPMENT

*R. Zhou,* ***L. Kroos****;*
*Michigan State University, East Lansing, MI*

After the forespore is engulfed by the mother cell membrane during *B. subtilis* sporulation, $\sigma^G$ in the forespore induces expression of SpoIVB, a serine protease believed to be secreted across the inner forespore membrane. This protease signals to a complex of proteins, including BofA, SpoIVFA, and SpoIVFB, that are sythesized in the mother cell and embedded in the outer forespore membrane. SpoIVFB is a metalloprotease that cleaves inactive pro-$\sigma^K$ to $\sigma^K$, which directs transcription of late genes in the mother cell. Cleavage of pro-$\sigma^K$ is thought to occur within the outer forespore membrane or near its surface on the mother cell side. It provided the first bacterial example of regulated intramembrane proteolysis (RIP), a mechanism whereby membrane-embedded proteases release transcription factors or polypeptide signals from membranes. To study the regulation of SpoIVFB, we have expressed different combinations of proteins in *E. coli.* This allowed us to show that BofA is the inhibitor of SpoIVFB. By adding additional proteins to the *E. coli* system, we now report that SpoIVFA enhances inhibition of SpoIVFB by BofA, and SpoIVB partially overcomes the extra inhibition brought about by SpoIVFA. This suggests that the SpoIVB serine protease targets SpoIVFA in complexes that inhibit pro-$\sigma^K$ RIP by SpoIVFB. We will present results of *in vitro* experiments with partially purified proteins, as well as pulse-chase, immunoprecipitation experiments with sporulating *B. subtilis*, that support this model. Using similar approaches, we have discovered that CtpB, a serine protease made under $\sigma^E$ control in the mother cell and potentially secreted across the outer forespore membrane into the space between the membranes surrounding the forespore, appears to cleave BofA after SpoIVB has cleaved SpoIVFA. Hence, RIP of pro-$\sigma^K$ appears to be regulated by a cascade of proteolysis in which SpoIVB from the forespore cleaves SpoIVFA, CtpB from the mother cell cleaves BofA, and SpoIVFB cleaves pro-$\sigma^K$.

# S4:5

## DEVELOPMENTAL COMMITMENT IN A BACTERIUM

***J. Dworkin****;*
*Columbia University, New York, NY*

We investigated developmental commitment during sporulation in *Bacillus subtilis*. Sporulation is initiated by nutrient limitation and involves division of the developing cell into two progeny, the forespore and the mother cell, with different fates. Differentiation becomes irreversible following division when neither the forespore nor the mother cell can resume growth when provided with nutrients. We show that commitment is governed by the transcription factors $\sigma^F$ and $\sigma^E$, which are activated in the forespore and the mother cell, respectively. We further show that commitment involves *spoIIQ*, which is under the control of $\sigma^F$, and *spoIIP*, which is under the control of both $\sigma^F$ and $\sigma^E$. In the presence of nutrients, the forespore can exhibit rod-like, longitudinal growth when SpoIIQ and SpoIIP are absent, whereas the mother cell can do so when SpoIIP alone is absent. We demonstrate that consistent with these observations, *spoIIP* is subject to two modes of expression: $\sigma^E$-directed transcription from a promoter located immediately upstream of the gene and $\sigma^F$-directed read-through transcription from the promoter of the adjacent upstream gene. Finally, *B. anthracis* contains two orthologs of *spoIIP* and when expressed in *B. subtilis* one is mother cell-specific and other is forespore-specific. Thus, developmental commitment of this single-celled organism, like that of the cells of complex, multi-cellular organisms, ensures that differentiation is maintained despite changes in the extra-cellular milieu.

# S4:6

## COORDINATING DEVELOPMENTAL GENE EXPRESSION IN MYXOCOCCUS XANTHUS

***A. G. Garza;***
*Syracuse University, Syracuse, NY*

When deprived of nutrients, *M. xanthus* initiates a complex developmental program that allows large groups of cells to migrate to aggregation centers and begin building multicellular fruiting bodies. Once a fruiting body is molded into its final shape, individual rod-shaped cells within this structure differentiate into dormant, spherical-shaped spores that are resistant to many forms of environmental stress. DNA microarray analysis has shown that at least 2000 *M. xanthus* genes are developmentally regulated. Recent studies suggest that NtrC-like transcriptional activators play a prominent role in regulating these large-scale changes in gene transcription. To date, 13 NtrC-like activators that are required for normal development have been uncovered. These proteins are used throughout the *M. xanthus* developmental cycle, and they have been linked to a variety of processes that are important for *M. xanthus* development, including gliding motility, cell-cell signal transduction, and the stringent response. We have shown that expression of many *ntrC*-like activator genes are developmentally regulated, and that their expression coincides with the time of development that they are required. Furthermore, we found that developmental expression of some activator genes is dependent on other NtrC-like activators. These findings suggest that *M. xanthus* may use a series of NtrC activators during fruiting body development in much the same way as *B. subtilis* uses a cascade of sigma factors during sporulation.

# S4:7

## STRUCTURAL PROTEINS INVOLVED IN DIFFERENTIATION OF STREPTOMYCETES

***D. Claessen***[1]*, W. de Jong*[2]*, G. Bucca*[3]*, V. Mersinias*[3]*, C. P. Smith*[3]*, L. Dijkhuizen*[2]*, H. A. Wösten*[4]*;*
[1]*University of Oxford, Oxford, UNITED KINGDOM,* [2]*University of Groningen, Haren, THE NETHERLANDS,* [3]*University of Surrey, Guildford, UNITED KINGDOM,* [4]*University of Utrecht, Utrecht, THE NETHERLANDS*

The soil habitat presents an array of challenges to its microbial community. The streptomycetes are important members of this ecosystem and they have evolved complex morphological and physiological adaptations that allow them to thrive in this environment. After a submerged mycelium has been formed filaments grow into the air to septate into chains of spores. Alternatively, substrate hyphae may grow over and attach to hydrophobic surfaces such as the leaf of a dead or living plant. We have identified and characterized two classes of structural proteins of *Streptomyces coelicolor*, called chaplins and rodlins, that are abundantly present on the outer surface of aerial hyphae and spores. These proteins turn out to be essential for formation of aerial structures as well as attachment of hyphae to hydrophobic surfaces.
A variety of strains have been described that are affected in the formation of aerial hyphae (*bld* mutants). In most of these mutants regulatory proteins are mutated. For instance *bldN* encodes an extracytoplasmic function sigma factor. It was shown that expression of the rodlin genes *rdlA* and *rdlB* was severely reduced in the *bldN* mutant. This suggested that expression is controlled (either directly or indirectly) by the BldN protein. If expression of the *rdlA* and *rdlB* genes was solely dependent on this (or any earlier) *bld* gene, one would expect these genes to be similarly expressed in the wild-type strain and in a strain lacking the chaplins (i.e. the Δ*chpABCDEFGH* strain). The latter strain hardly forms aerial hyphae, but otherwise grows like the wild-type. Interestingly, *rdl* expression was strongly reduced in colonies of the Δ*chpABCDEFGH* strain, although the *rdl* expression level *per aerial hypha* was identical to the wild-type strain. These data strongly suggest that hyphae sense their presence in the air and, as a consequence, activate aerial hyphae specific genes, two of which being the *rdl* genes. This novel sensing mechanism, which we called the Skyscraper pathway, may play a key role in development of streptomycetes.
Currently, we are using DNA microarrays to compare global gene expression in the wild-type and Δ*chpABCDEFGH* strain to identify genes that are subject to regulation by the Skyscraper pathway. These genes possibly encode proteins involved in formation of aerial hyphae. The results so far have identified several interesting genes that will be further characterized.

# S4:8

## THE CHAPLINS OF *STREPTOMYCES COELICOLOR*: IMPORTANT DEVELOPMENTAL PROTEINS THAT ARE ASSOCIATED WITH THE TAT SECRETION PATHWAY

***M. Elliot***[1]*, D. Widdick*[2]*, T. Palmer*[2]*, M. Buttner*[2]*;*
[1]*McMaster University, Hamilton, ON, CANADA,* [2]*John Innes Centre, Norwich, UNITED KINGDOM*

The filamentous bacterium *Streptomyces coelicolor* differentiates by forming specialised spore-bearing aerial hyphae. We have identified a family of eight proteins (ChpA-H) that are essential for the formation of aerial hyphae. The members of the 'chaplin' family are all secreted via the Sec secretion pathway, and share a conserved hydrophobic region (the 'chaplin domain') that is rich in beta-sheet secondary structure, and has two highly conserved Cys-residues. Five of the chaplins (ChpD-H - the short chaplins) consist exclusively of the chaplin domain, while the other three chaplins (ChpA-C -

the long chaplins) have two chaplin domains and a predicted C-terminal sorting signal which targets them for covalent attachment to the cell wall peptidoglycan by the sortase enzyme. We have found that the five short chaplins are also localised to the cell wall, and that deletion of all the chaplin genes results in a strain that is unable to raise an aerial mycelium. This suggests a critical role for the chaplins in aerial morphogenesis. Intriguingly, we have been unable to knock-out one of the chaplin genes (*chpE)* in a wild type genetic background, suggesting that not only are the chaplins important for development, but that ChpE is crucial for viability as well. It has, however, been possible to create a *chpE* null mutant in the absence of the other chaplin genes. This implies that the presence of the other chaplins, in the absence of ChpE, is detrimental for growth and viability. Furthermore, we have identified several *chpE* suppressor mutations in the *tatB* gene, which encodes an essential component of the twin arginine translocation (Tat) secretion pathway, suggesting that there is a unique connection between the Tat secretion system, the chaplins, and development in *S. coelicolor*.

# S5:1

## DEVELOPMENT OF *RHIZOBIUM LEGUMINOSARUM* BACTEROIDS IN PEA NODULES

***Philip Poole***

*School of AMS University of Reading UK*

*Rhizobium leguminosarum* biovar *viciae* is able to enter into a symbiotic interaction with pea plants resulting in the formation of root nodules by the plant. Within the nodules the free-living rhizobia grow down infection threads, which keep the outside plant cells, until they reach the nodule cortex where they are engulfed by plant cells by endocytosis. The rhizobia then differentiate into nitrogen fixing bacteroids, which no longer appear to be capable of dividing and cannot be recovered from nodules. As the free-living rhizobia reach the II-III interzone of the nodule they breakdown large polyhydroxybutyrate stores, which fuel the development of bacteroids. We have proposed this as a carbon burst model of development.

It has been known for several decades that the plant provides dicarboxylic acids to the bacteroid as a carbon and energy source and in return the plant is provided with ammonium from reduced $N_2$. The plant then assimilates the ammonium into the amino acids glutamine and asparagine, which are exported from the root to the shoot. However, we have recently shown that an amino acid cycle also operates between the bacteroid and plant. An amino acid such as glutamate or possibly GABA is provided by the plant and the bacteroid converts this to alanine and secretes it back to the plant cytosol. We have therefore proposed the operation of a GABA/alanine cycle. This suggests a complex mutualism between the two partners where both are completely dependent on one another. Bacteroids appear to act as plant organelles and this suggest an interesting intermediate in the formation of true organelles such as mitochondria and chloroplasts. In this context it is particularly interesting that they all belong to the α-proteobacterial group.

# S5:2

## SEQUENTIAL BACTERIAL-HOST SIGNALING LEADS TO LIGHT ORGAN DEVELOPMENT AND SYMBIOSIS

***E. G. Ruby;***
*University of Wisconsin, Madison, WI*

It has become increasingly clear that all animal and plant species live in association with specific microbial symbionts whose activities are essential to normal health and development of the host. While many of these microbes exist as complex consortia of tens or hundreds of species, other symbioses, like the association of Vibrio fischeri in the light-emitting organs of certain squids and fishes, are strictly monospecific. Such simple systems provide an opportunity to more easily examine signaling mechanisms that may underlay the development of other more complex microbial symbioses. The squid-vibrio association initiates at each host generation when V. fischeri cells present in seawater inoculate the nascent light organs of juvenile squids, an event that triggers a coordinated developmental program of biochemical and morphological changes in both partners. These changes begin within the first few hours of the association and, in the bacterium, include modifications of behavior and metabolism that are controlled by chemotaxis, quorum sensing, and other signaling systems. One of the earliest signals is chitobiose, which is produced by the squid and serves as a chemotactic signal that facilitates the initial steps in colonization of the nascent light organ. Once colonization begins, a temporal program of gene expression is initiated in the bacterium by the sequential activity of two acyl-homoserine lactone inducers, resulting in the modification of motility behavior, luminescence and other activities in the symbionts. These events are accompanied by the expression of bacteria-produced signals that trigger a congruent development of the tissues of the light organ, resulting in a functional structure that is designed to maintain a specific, homeostatic infection throughout the lifetime of the host. While this highly structured association develops only between its specific species pair, it has evolved by the recruitment and modification of activities like chitin production and utilization, quorum sensing, and immune response that are common to all marine vibrios and invertebrates.

## S5:3

### THE CYANOBACTERIAL CIRCADIAN CLOCK

***S. S. Golden;***
*Texas A&M University, College Station, TX*

Cyanobacteria, like diverse eukaryotes, possess intrinsic timing systems called circadian clocks that allow cells to coordinate physiological processes with the predictable daily patterns of environmental fluctuations on Earth. The circadian oscillator of the cyanobacterium *Synechococcus elongatus* (PCC 7942) generates daily rhythms of gene expression throughout its genome; thus, gene expression yields a tractable real-time monitor of circadian rhythmicity via bioluminescence expressed from luciferase reporters. The rhythm of bioluminescence driven by *S. elongatus* promoters provides phenotypes amenable to mutant analysis, exhibited as absence of rhythmicity, or changes in the time required for one complete cycle (circadian period), the amplitude of the oscillation, or the timing of peaks relative to an environmental cue (circadian phase). Genetic, biophysical, and biochemical approaches have yielded insights into the nature of the circadian clock of this cyanobacterium and developed it as a system in which it will be possible to achieve comprehensive understanding of the circadian mechanism. The goal is to understand how the cyanobacterial cell organizes its internal oscillator for 24-h timekeeping, transmits temporal information to clock-controlled genes, and synchronizes the clock with the external environment. Molecular structures have been solved for the key circadian oscillator components, a functional genomics project is nearing global saturation mutagenesis to achieve identification of all circadian-related loci, and various lines of research are tying the clock to fundamental cellular functions. Work from several labs has identified genes whose functions are critical for generating, setting, or relaying circadian rhythms. The three-gene *kai* locus (*kaiA*, *kaiB*, *kaiC*) is essential for circadian rhythmicity. The *cikA* gene encodes a histidine protein kinase that is necessary for resetting the phase of rhythms from the clock in response to an environmental cue. An Fe-S protein called LdpA senses changes in redox state of the cell as an indirect measure of the light environment and modulates circadian period. The histidine protein kinase SasA physically interacts with KaiC and is important for relay of temporal information to downstream genes. The picture that is emerging of the circadian timekeeping mechanism is of a multiprotein, multimeric, molecular machine composed of proteins (KaiA, KaiB, KaiC, SasA, and others) whose domains exhibit twists on common themes. The three Kai proteins intrinsically possess the ability to keep near 24-h time, as their interactions can set up a circadian rhythm of KaiC autophosphorylation *in vitro* in the presence of ATP. Other proteins likely tie the clock to the functions it controls and provide signal transduction functions to ensure that the cellular clock stays in register with its environment.

## S5:4

### PREDATORY *BDELLOVIBRIO* : GENE EXPRESSION FROM ATTACK PHASE TO THE BDELLOPLAST

***R. E. Sockett;***
*University of Nottingham, Nottingham, UNITED KINGDOM*

*Bdellovibrio bacteriovorus* are small, motile, flagellate, predatory delta proteobacteria that penetrate, reseal and internally consume other Gram-negative bacteria, replicating within their periplasm in a state called the bdelloplast, before lysing them and attacking further prey. *Bdellovibrio* are "lone hunters" unlike the "pack" hunting *Myxobacteria* to which they are phylogenetically related. We have shown that active flagellar motility is required for locating prey-rich regions and for efficient predation of prey populations, and we are testing the hypothesis that aflagellate *Bdellovibrio* directly applied to a prey surface can enter it. Genome sequencing of *B. bacteriovorus* HD100 revealed myriad hydrolytic enzyme genes that must be co-regulated for efficient degradation of prey. *Bdellovibrio* effectively hydrolyse prey macromolecules to monomers, take these up and repolymerize them. We are studying the expression of subsets of these genes at timepoints during the predation cycle by RTPCR and the timing of expression reveals some of the organisation of prey degradation. In the bdelloplast the *Bdellovibrio* grow co-encytically in the periplasm of another bacterium - this brings with it a requirement for developmental responses to periplasmic biochemical conditions. We have studied the expression of genes that potentially afford *Bdellovibrio* the opportunity to use nitrate/ nitrite as terminal electron acceptors and are currently studying the effect of inactivating these genes on viability in the periplasm. Predatory life brings with it novel demands on the core metabolism of the bacterium; one of the surprises of the *Bdellovibrio* genome was that it is of the size and complexity of a non-predatory bacterium. Thus *Bdellovibrio* may be seen as a saprophyte that "learned" to invade other bacteria, not a true parasite. A collaboration with Ian Henry and Paul Sharp has examined *in silico*, the extent to which the predatory lifestyle may have brought requirements for altered *Bdellovibrio* expression of "core metabolic" genes in comparison to that seen for saprophytic bacteria. We have followed this up with RTPCR expression studies and these combine to give us a glimpse of the "cost-benefit" trade-offs of the predatory lifestyle for *Bdellovibrio.*

# S5:5

## IDENTIFICATION AND CHARACTERIZATION OF FIVE SMALL RNAS THAT CONTROL QUORUM SENSING IN VIBRIO HARVEYI

***K. Tu**, B. Bassler;*
*Princeton University, Princeton, NJ*

Quorum sensing is a strategy bacteria use to collectively regulate gene expression and, at a higher level, coordinate group behavior. Quorum sensing involves the production and secretion of extracellular signaling molecules known as autoinducers (AIs). The accumulation of a threshold concentration of these molecules in the environment enables bacteria to detect one another's presence, and thereby conduct a "census" of their population. Quorum sensing in the marine bacterium Vibrio harveyi involves a multi-channel, two-component phosphorelay signal transduction circuit that regulates light (luciferase) production and many other genes. Sensory information from two independent quorum-sensing systems converges onto a conserved sigma-54-dependent response regulator called LuxO. When LuxO is phosphorylated (LuxO~P, low cell density), it activates the expression of a putative repressor that destabilizes the mRNA encoding the master transcriptional regulator LuxR. In the closely related species Vibrio cholerae, a combination of genetic and bioinformatics studies revealed this repressor to be the RNA chaperone Hfq together with four small regulatory RNAs (sRNAs) called Qrr1-4 (Quorum Regulatory RNA). We predict that Hfq and multiple sRNAs also act as the repressor in V. harveyi. However, the sRNAs have not been identified because the V. harveyi genome has not been sequenced. A novel strategy based on differential fluorescence induction was developed to identify the analagous regulatory sRNAs in V. harveyi. This screen enabled the isolation of three V. harveyi sRNA promoters (Qrrs2,3,4), and studying their expression has provided insight into their regulation. Other methods allowed the identification of Qrr1 and Qrr5 in V. harveyi, although interestingly, these promoters do not appear to be regulated by quorum sensing. However, overexpression of either of these two sRNAs shows that they act on luxR mRNA. These findings suggest that all five sRNAs function to regulate levels of LuxR in V. harveyi, although the regulatory mechanism controlling each one is different.

# S6:1

## SIGNALING THE REGULATORY NETWORK FOR *MYXOCOCCUS XANTHUS* FRUITING BODY DEVELOPMENT

*Lars Jelsbak; **Dale Kaiser***

*Departments of Biochemistry and Developmental Biology, Stanford University, Stanford, CA*

In response to starvation, *Myxococcus xanthus* initiates a developmental program that results in the formation of fruiting bodies containing 100,000 spores. Many developmentally regulated genes in *M. xanthus* are transcribed from sigma-54 promoters, and each of those transcriptions requires a specific enhancer binding protein. The recently completed genome shows more than 50 different genes encoding $\sigma^{54}$-dependent enhancer binding proteins (EBP). The genome also has 100 different genes encoding serine/threonine protein kinases (STPK), which are more common in eukaryotes than bacteria. Twelve of the EBP have forkhead-associated N-terminal sensory domains having the capacity to recognize phospho-threonine residues in other proteins. A null mutation in one of the forkhead EBP genes, *Mx4885*, caused a cell autonomous aggregation and sporulation defect; an in-frame deletion of the forkhead domain alone caused the same defect. The altered pattern of developmental gene expression and protein production in the mutant implies that Mx4885 lies at a unique position on the response pathway to the morphogenetic C-signal; it lies just downstream of C-signal induced synthesis of the FruA response regulator on that pathway. Serine/threonine protein kinases in other organisms respond to extracellular signals. Their incorporation into pathways that regulate development shows how an expanded sensitivity to extracellular signals may have evolved to enable myxococcus to build species specific fruiting bodies.

# S6:2

## SIGNALING IN HETEROCYST DIFFERENTIATION

***J. W. Golden**;*
*Texas A&M University, College Station, TX*

Many filamentous cyanobacteria fix nitrogen in specialized terminally differentiated cells called heterocysts. Anabaena (Nostoc) sp. strain PCC 7120 produces a developmental pattern of single heterocysts separated by 10 to 15 photosynthetic vegetative cells. Several genes have been identified that are involved in the regulation of heterocyst differentiation and pattern formation including: ntcA, hetR, hetN, patA, and patS. NtcA is a transcription factor involved in global regulation related to carbon/nitrogen balance, and is

essential for multiple aspects of heterocyst development. HetR has protease and DNA-binding activities, and is a master regulator of heterocyst differentiation. The patS gene encodes a 13 (or 17) amino acid peptide that is thought to serve as a diffusible cell-to-cell signal that contributes to the regulation of heterocyst pattern by lateral inhibition. Overexpression of patS inhibits heterocysts, and a patS deletion mutant forms multiple contiguous heterocysts and an abnormal pattern. A synthetic peptide representing the C-terminal 5 amino acids of PatS (PatS-5, RGSGR) inhibits heterocyst development, and PatS-5 inhibits HetR's DNA-binding activity in vitro. The current data support a model in which PatS signaling is important for the initial resolution of pairs and clusters of differentiating cells by inhibiting HetR. However, factors other than PatS also are involved in regulating heterocyst pattern, and these include signaling and regulation related to PatA and HetN, and nitrogen compounds supplied by heterocysts. We are using several approaches to characterize the PatS signaling pathway. A set of patS minigenes encoding only the last 4, 5, 6, 7, and 8 codons were constructed and all except the smallest suppressed heterocyst development. A GFP-PatS-5 fusion protein, a PatS-6His fusion, and two Anabaena open reading frames (other than patS and hetN) that encode an internal RGSGR sequence all suppress heterocysts when overexpressed in vegetative cells. These data are consistent with the PatS receptor being HetR, which is located in the cytoplasm. Genetic screens related to PatS signaling have identified several genes. For example, strains containing a hetR R223W allele fail to respond to PatS or other pattern formation signals. Overexpression of the R223W allele results in a lethal phenotype in which all cells differentiate a few days after nitrogen step-down. Another gene, orf93, inhibited heterocyst differentiation when overexpressed, even in a patS mutant strain, and inactivation of orf93 led to an increased frequency of heterocysts. A transcriptional fusion with a gfp reporter gene showed that orf93 is up-regulated in differentiating proheterocysts. The orf93 product does not show similarity to known proteins so understanding its role in heterocyst development will require identifying its partners.

# S6:3

## SPATIAL REGULATION OF A SIGNALING PATHWAY

***Judy Armitage**, University of Oxford, Oxford, UK*

The photoheterotrophic bacterium *Rhodobacter sphaeroides* expresses two complete chemosensory pathways, encoded by two different operons. The $CheA_2$, $CheW_2$, $CheW_3$ and $CheR_2$ proteins encoded by one operon (Che $Op_2$) localise to the cell poles with the transmembrane chemoreceptors, while $CheA_3$, $CheA_4$, $CheW_4$ and $CheR_3$ (encoded by Che $Op_3$) all localise with the cytoplasmic chemoreceptors to a cluster in the mid point of the cell. The expression of Che $Op_2$ is regulated by environmental conditions while Che $Op_3$ is expressed at a relatively constant level under all conditions. Both sets of proteins are required for chemotaxis and while there is some crosstalk between the phosphotransfer proteins *in vitro*, there is no evidence that they can complement *in vivo*-why is this the case? All the genes for the chemosensory proteins have been replaced in the genome by either N or C terminal CFP or YFP fusions and checked for functionality. Using DIC microscopy we showed that localisation of the chemosensory proteins to the cell poles is dependent on $CheA_2$ and $CheW_2$. Deletion of either lead to the MCPs becoming delocalised and the other protein diffuse in the cytoplasm. Interestingly, even when diffuse in the cytoplasm, $CheA_2$ and $CheW_2$ showed no association with the cytoplasmic chemosensory cluster. Unlike the polar cluster, formation of the cytoplasmic cluster does not depend of either $CheA_3$ or $CheA_4$, both of which can be deleted either independently or together without altering the structure of the cytoplasmic cluster. Deletion of $CheW_4$ caused partial delocalisation of the cluster, as did deletion of either of the cytoplasmic chemoreceptors TlpL or TlpC. However, deletion of the receptor TlpT caused complete delocalisation of the other proteins within the cluster. This suggests that in the case of the cluster of cytoplasmic chemosensory proteins, the receptor TlpT acts as the scaffold onto which the other chemosensory proteins build to form the signaling pathway. Again, when diffuse the cytoplasmic proteins do not appear to localise to the polar clusters, even when homology is high. The two chemosensory pathways are localised within the cell and apparently do not cross-talk. Interestingly, encoded between $CheW_4$ and TlpT on Che $Op_3$ is a protein with homology to the ParA family of plasmid segregating proteins. The cytoplasmic cluster localises to the mid-cell but before division a second cluster becomes visible and the two clusters segregate to mid cell of the daughter cells. Cephalexin induced filaments have evenly spaced clusters at approximately one cell length separation. Deletion of the ParA homologue or mutation of the putative ATPase resulted in cells with a single, large, randomly positioned cluster, even in filaments. These data suggest a protein partitioning factor regulating the position of the cytoplasmic cluster of chemosensory proteins.

# S6:4

## SENSING DNA DAMAGE UPON ENTRY INTO SPORULATION IN *BACILLUS SUBTILIS*

***S. Ben-Yehuda**, M. Bejerano-Sagie, Y. Oppenheimer-Shaanan, I. Berlatzky;*
*The Hebrew University of Jerusalem, Jerusalem, ISRAEL*

Checkpoint mechanisms block cell cycle progression or development in response to DNA damage to provide cells with time to repair the DNA lesions and thus to ensure that genomic integrity is maintained. Sensing and repairing lesions are particularly acute in *Bacillus subtilis* cells entering the developmental process of sporulation, as damaged DNA may prevent the cells from completing spore morphogenesis. Indeed, in the presence of DNA damaging agents, the sporulation process is inhibited at an early stage, prior to asymmetric cell division. However, the mechanism by which sporulating cells sense and respond to genome damage is not

well understood. We report the identification of a novel protein DisA (for DNA integrity surveillance) that is required to delay the progression of sporulation in response to DNA damage. DisA encodes a nonspecific DNA binding protein, which is induced early in sporulation as well as under variety of stress conditions. Under these conditions, a DisA-GFP fusion forms foci that colocalize with the DNA. Cells mutant for DisA enter sporulation prematurely in the presence of chromosomal lesions, resulting in fewer viable spores compared to wild-type cells. Conversely, overproduction of the protein causes a severe delay in the initiation of sporulation. We propose a model in which DisA scans the chromosome for damages. Once a lesion is identified, DisA induces a cellular response that culminates in a temporary block in sporulation. Thus, DisA prevents cells from entering spore morphogenesis under conditions in which they cannot form mature spores successfully and, hence, may perish trying.

# S6:5

## BIOCHEMICAL AND GENETIC IDENTIFICATION OF A C-DI-GMP BINDING MOTIF

***B. Christen**, M. Christen, U. Jenal;*
*University of Basel, Basel, SWITZERLAND*

Cyclic di-guanosine monophosphate (c-di-GMP) has recently been shown to be a novel secondary messenger involved in regulating bacterial motility and community behavior. An increasing number of genetic studies in different bacteria showed that c-di-GMP controls the developmental switch between a motile, planktonic and a surface attached biofilm mode. Diguanylate cyclases (DGC), the enzymes that catalyze the formation of c-di-GMP from GTP, are widespread in bacteria and the presence of a large number of paralogs in many bacterial species poses the question of how DGCs from parallel signal transduction pathways are controlled.
We use *Caulobacter crescentus* PleD, a multi domain response regulator protein with a DGC (GGDEF) output domain as a model to investigate controlled synthesis of c-di-GMP in bacterial cells. We have found that the diguanylate cyclase activity of PleD is activated in response to phosphorylation but in addition is subject to direct feedback inhibition by its own product. The recently solved crystal structure of PleD shows that c-di-GMP does not only bind to the active site (A-site) of the DGC domain, but also to a putative allosteric binding site (I-site). Using site-directed mutagenesis in combination with UV cross-linking and tryptic digest experiments, we confirmed the existence of the I-site in solution. Here we present evidence that an additional *C. crescentus* GGDEF protein, CC3285, exhibits DGC activity and is regulated by the same c-di-GMP feedback inhibition mechanism. To probe the nature of the I-site binding pocket, we generated a CC3285 I-site mutant library by randomized but targeted mutagenesis and used a novel genetic screen to select for highly active mutants that had lost feedback control but maintained catalytic DGC activity. The results will be discussed with respect to the ligand binding properties and the regulatory mechanisms involved in allosteric control of DGC proteins. This is the first binding motive identified for the novel secondary messenger c-di-GMP. Our results and the observation that the amino acids of the I-site pocket are conserved in a large fraction of the known GGDEF proteins, argue that allosteric product inhibition is a general control mechanisms of bacterial DGCs.

# S6:6

## SENSING SIGNALS THROUGH THE FRZ SIGNAL TRANSDUCTION SYSTEM OF MYXOCOCCUS XANTHUS

***D. R. Zusman**, D. Astling, J. Lee, V. H. Bustamante, I. Martinez-Flores, A. Scott;*
*University of California, Berkeley, CA*

The Frz system is a chemosensory signal transduction pathway that regulates both vegetative swarming and developmental aggregation in Myxococcus xanthus by controlling the reversal frequency of cells. Cell reversal in M. xanthus allows cells to alter their movements and show directed motility in response to chemical gradients. FrzCD, unlike most methylated chemotaxis protein (MCP) receptors, is a cytoplasmic receptor that lacks the periplasmic and HAMP domains characteristic of most bacterial chemoreceptors. How then are signals sensed by this receptor? Deletion analysis showed that this receptor can function almost normally without its entire N-terminal domain; however, localization of the receptor is altered in these mutants. Site directed mutagenesis of potential methylation sites within FrzCD showed that mutations at some sites were most inhibitory to vegetative swarming and other sites to development. FrzF, the Frz system specific methyltransferase, is much larger than CheR from E. coli, containing an additional domain with three tandem TPR repeats. We constructed a mutant with an in-frame deletion in the TPR repeats and found that it was blocked in development but not vegetative swarming. We purified FrzF and found that it could methylate FrzCD in vitro when incubated with S-adenosyl methionine. We also purified FrzF containing the TPR repeat deletion and tested its ability to methylate FrzCD. Surprisingly, this mutant enzyme hypermethylated FrzCD in vitro. These results suggest that the TPR domain of FrzF modulates the activity of the methylating domain and that this regulation is required for developmental aggregation but not vegetative swarming.

# S6:7

## THE DIF CHEMOSENSORY PATHWAY IS DIRECTLY INVOLVED IN PHOSPHATIDYLETHANOLAMINE SENSORY TRANSDUCTION IN *MYXOCOCCUS XANTHUS*

***P. J. Bonner***[1]*, Q. Xu*[2]*, W. P. Black*[2]*, Z. Li*[2]*, Z. Yang*[2]*, L. J. Shimkets*[1]*;*
*[1]University of Georgia, Athens, GA, [2]Virginia Polytechnic Institute and State University, Blacksburg, VA*

*Myxococcus xanthus* cells glide on solid surfaces and are chemotactically excited by certain phosphatidylethanolamine species, resulting in a suppression of reversals that is analogous to the suppression of tumbles by chemoattractants in flagellated bacteria. The *dif* gene cluster consists of six genes, *difABCDEG,* of which five encode proteins homologous to known chemotaxis proteins. DifA, a methyl-accepting chemotaxis protein (MCP), DifC, a coupling protein (CheW homolog), and DifE, a histidine kinase (CheA homolog), are required for the biosynthesis of fibrils, an extracellular polysaccharide matrix decorated with protein. Chemotaxis to 1,2-*O*-Bis[11-(*Z*)-hexadecenoyl]-*sn*-glycero-3-phosphatidylethanolamine (16:1 PE) requires fibrils, and although previous work has shown that *difA* and *difE* mutants do not excite, these results do not distinguish between a dependence on fibrils or a direct role in chemosensory transduction. Here we provide evidence that the Dif chemosensory pathway is directly involved in PE sensory transduction, in addition to its role in fibril biogenesis. First, excitation and adaptation to 16:1 PE requires all of the *dif* genes, including *difBDG,* which are not essential for fibril biogenesis. Deletion of *difD,* which encodes a CheY homolog, prevents excitation to 16:1 PE. *difB*, which encodes a protein of unknown function, and *difG*, which encodes a CheC homolog, have roles in adaptation to 16:1 PE. Second, a specific residue within the first methylation domain of DifA is required for excitation to 16:1 PE but not fibril biogenesis. Analyses of the chemotactic responses of cells containing a chimeric NarX-DifA chemoreceptor indicate that transmembrane signaling is required for fibril biosynthesis, but not excitation or adaptation to 16:1 PE. DifA may integrate unknown stimuli for fibril biogenesis and 16:1 PE chemotaxis through different domains. Third, a portion of the Dif pathway is required for excitation to dioleoyl PE (18:1 PE) although the response is not dependent on fibrils. Mutations in *difD* or *difE* prevent excitation with 18:1 PE indicating a direct role for the encoded histidine kinase-response regulator pair. Sensory perception of 18:1 PE does not require DifA or DifC suggesting that a DifA-independent sensing mechanism exists. Results with site-directed *difD* mutations that are nonphosphorylatable or mimic the phosphorylated state of DifD suggest that Dif-mediated chemosensory transduction is similar to the enteric model. Attractant binding is predicted to prevent phosphorylation of DifD, resulting in increased reversal periods.

# S6:8

## ANALYSIS OF SIGNAL TRANSDUCTION AND CHEMOTAXIS IN MYXOCOCCUS XANTHUS

***J. R. Kirby;***
*Georgia Institute of Technology, Atlanta, GA*

Signal transduction is critical for the regulation of developmental programs in bacteria. While several signals have been identified in the control of fruiting body formation in Myxococcus xanthus, many more signal transduction proteins have been identified through classical techniques and bioinformatics.
Analysis of the completed genome of M. xanthus reveals the presence of 554 proteins comprising a combined total of more than 400 one-component and two-component signal transduction systems. Among the two-component systems are eight che systems, each defined by the presence of a cheA gene. This is the largest number of che genes in any prokaryote to date. M. xanthus also possesses 21 chemoreceptors, a number that is typical for the proteobacteria.
Until recently, chemotaxis systems had only been thought to regulate motility rather than other cellular functions such as gene expression. Results now suggest that M. xanthus, Rhodospirillum centenum, and Pseudomonas aeruginosa all utilize paralogous chemosensory systems to regulate other aspects of cell physiology.
Genome context and molecular characterization have led to a better understanding of the possible roles of many of the chemosensory systems in M. xanthus. Results from our analysis of Che3, Che5, and Che8 indicates that these systems do not affect motility but regulate timing of development by unknown mechanisms. Additionally, all 8 cheA mutants display unique phenotypes under both vegetative and developmental conditions, indicating that each system regulates unique aspects of the complex life cycle in M. xanthus.

# S7:1

## INVESTIGATING *STREPTOMYCES* DEVELOPMENTAL BIOLOGY IN THE GENOMIC ERA.

***K. Chater;***
*John Innes Centre, Norwich, UNITED KINGDOM*

In the main biomass accumulation phase of *Streptomyces coelicolor,* a single spore germinates to form a multicellular mycelium, consisting of multigenomic compartments that extend by branching and tip growth. Under laboratory conditions, this "substrate mycelium" then supports the

growth of specialised reproductive branches into the air, partially by reusing its own biomass. The aerial branches continue to extend until they contain often as many as 100 genomes. The long apical compartment then undergoes synchronous multiple septation to generate unigenomic prespore compartments.These undergo wall thickening and rounding up to become chains of spores. Many regulatory genes and some structural proteins involved in this complex reproductive programme have been identified and some of their interactions have been studied. Coupling this knowledge with the growing number of available genome sequences from other streptomycetes and related actinomycetes, all developmentally and genetically less complex, we can begin to consider how *Streptomyces* development evolved. Many of the developmental regulatory genes appear to be specific to streptomycetes (although members of the same gene families - paralogues - are often widespread). However, among both the *bld* genes involved in the formation of aerial mycelium and the *whi* genes needed for aerial hyphae to develop into pigmented spore chains, some genes do have apparent orthologues in other actinomycetes or even further afield. In the special case of *bldA,* which encodes the only tRNA for the rare leucine codon UUA, it is straightforward to identify potential target genes in the sequenced genomes of *S. coelicolor* and *S. avermitilis*. Remarkably, very few of the TTA-containing genes are conserved between the two species. These conserved genes may include a core set with major roles in all species, while the non-conserved target genes probably have species-specific adaptive functions. Using functional genomics, some indirect effects of *bldA* mutation on *S. coelicolor* have been observed, and the mechanism of some of these effects has been elucidated.

## S7:2

## RODK, AN UNUSUAL HYBRID HISTIDINE PROTEIN KINASE, REGULATES MULTIPLE STEPS DURING FRUITING BODY FORMATION IN *MYXOCOCCUS XANTHUS*

***Lotte Søgaard-Andersen***
*Max Planck Institute for Terrestrial Microbiology, Marburg, Germany*

In *M. xanthus* the ordered execution of fruiting body formation crucially depends on intercellular signalling systems, which stimulate and coordinate aggregation and sporulation temporally and spatially. Recently, it has become evident that several signal transduction systems act to inhibit fruiting body formation. Thus, the emerging picture of the design of the regulatory network that govern fruiting body formation is one in which the outputs of positively and negatively acting systems are integrated to determine the overall outcome.

We describe an unusual hybrid histidine protein kinase, which is important for spatially coupling cell aggregation and sporulation during fruiting body formation. A *rodK* mutant makes abnormal fruiting bodies and spores develop outside the fruiting bodies. RodK is a soluble, cytoplasmic protein, which contains an N-terminal sensor domain, a histidine protein kinase domain and three receiver domains. RodK is present in vegetative cells and remains present until the late aggregation stage, after which the level decreases in a manner that depends on the intercellular A-signal. Genetic evidence suggests that RodK may regulate multiple temporally separated events during fruiting body formation including stimulation of early developmental gene expression, inhibition of production of the intercellular A-signal, and inhibition of the intercellular C-signal transduction pathway. *In vitro* phosphorylation assays showed that RodK possesses kinase activity. Kinase activity is essential for RodK function *in vivo*. *In vitro* phosphorylation assays provided evidence for phosphotransfer to at least one of the receiver domains. By systematically substituting the conserved, phosphorylatable Asp residues in the three receiver domains, genetic evidence is provided that each receiver domain has a distinct function in fruiting body formation and that the function of each receiver domain depends upon phosphorylation. We speculate that the kinase activity of RodK undergoes a change during development, which is reflected in the phosphorylation status of the three receiver domains, and that stepwise changes in the phosphorylation status of the three receiver domains allows RodK to regulate temporally separated events. Loss of RodK function appears to result in a collapse of the developmental program and as a consequence the spatial coupling of aggregation and sporulation is lost.

## S7:3

## METABOLOME ANALYSES OF MYXOCOCCUS XANTHUS REVEAL BIOMARKERS OF MYXOBACTERIAL DEVELOPMENT

***H. B. Bode***[1]*, M. Ring*[1]*, R. M. Kroppenstedt*[2]*, S. Schulz*[3]*, D. Kaiser*[4]*, G. Schwär*[1]*;*
*[1]Saarland University, Saarbrücken, GERMANY, [2]German National Resource Centre for Biological Material (DSMZ), Braunschweig, GERMANY, [3]Technical University Braunschweig, Braunschweig, GERMANY, [4]Stanford University, Stanford, CA.*

With their complex life cycle culminating in the formation of myxospores in sophisticated fruiting bodies (1), myxobacteria are an ideal model to investigate the physiological changes occurring during the underlying processes. We have focused on metabolome analyses using gas chromatography (GC) and high performance liquid chromatography (HPLC) coupled to mass chromatography (MS) in order to get a better understanding of the biochemistry behind myxobacterial fruiting body formation and sporulation. Especially GC/MS has a proven track record as a powerful tool to analyze complex biological samples consisting of several low molecular weight compounds such as primary and intermediary metabolites (i.e. amino acids, sugars, lipids). Using this technique we were able to identify known biomarkers of

myxobacterial development (accumulation of the A-signal mixture of amino acids (2) during early development and trehalose in the spores (3)). We have also identified previously unknown biomarkers. A specific increase during development of two unusual etherlipids and a fatty acid aldehyde were observed and shown to be spore-specific (a 40-fold increase was observed in the spores compared to vegetative cells). Analysis of several fruiting body-deficient mutants revealed a strongly reduced amount of these lipids in esg (E-signal defective (4)) mutants that have a reduced amount of isovaleryl-CoA (5,6). Isovaleryl-CoA is the starting unit for iso-fatty acids, the dominant fatty acid family in myxobacteria (7). Furthermore, esg mutants were rescued by the addition of isovaleric acid and the corresponding fatty acid and lipid derivatives derived thereof. Due to the high amount of the lipid compounds produced during development we suggest their major function to be structural to protect myxospores from hydrolysis. However, an additional signaling function could not be excluded. Finally we tested if mutants depleted in the amount of non-iso fatty acids (the second important fatty acid family in M. xanthus), show developmental defects as well. Whereas no difference between such mutants and the wildtype could be observed, reduction of the amount of iso-fatty acids resulted in severe developmental defects (see above) furthermore indicating the important role of iso-fatty acids for myxobacteria.

1. Kaiser, D. (2004) Annu Rev Microbiol 58, 75-98
2. Kuspa, A. et al. (1992) J Bacteriol 174, 3319-3326
3. McBride, M. J., and Zusman, D. R. (1989) J Bacteriol 171, 6383-6386
4. Toal, D. R. et al. (1995) Mol Microbiol 16, 177-189
5. Mahmud, T., Bode, H. B., Silakowski, B., Kroppenstedt, R. M., Xu, M., Nordhoff, S., Höfle, G., and Müller, R. (2002) J Biol Chem 277, 23768-32774
6. Mahmud, T., Wenzel, S. C., Wan, E., Wen, K. W., Bode, H. B., Gaitatzis, N., and Müller, R. (2005) ChemBiochem 6, 322-330
7. Bode, H., Dickschat, J. S., Kroppenstedt, R. M., Schulz, S., and Müller, R. (2005) J Am Chem Soc 127, 532-533

# S7:4

## INHIBITION OF BIOFILM FORMATION BY BACILLUS SUBTILIS THROUGH HIGH LEVELS OF SPO0A~P

*M. A. Hamon, C. Lee, V. T. Nguyen,* ***B. A. Lazazzera****; UCLA, Los Angeles, CA.*

Biofilms are a ubiquitous, multicellular lifestyle exhibited by bacteria, in which they are adherent to a surface and encased in a matrix. How biofilm formation is coordinated with the other lifestyles exhibited by bacteria is poorly understood. Here, we present investigations of how *Bacillus subtilis* coordinates biofilm formation and sporulation. The initiation of biofilm formation and sporulation both require the phosphorylated form of the response regulator Spo0A (Spo0A~P). However, biofilm formation and sporulation often do not occur under the same growth conditions. The role of Spo0A in biofilm formation is to repress *abrB*, and several lines of evidence indicate that low levels of Spo0A~P are sufficient to repress *abrB*. In contrast, high levels of Spo0A~P are known to activate the sporulation pathway. Thus, the lack of high levels of sporulation under conditions that lead to efficient biofilm formation could be explained by cells having low levels of Spo0A~P. These data were not sufficient to explain the lack of biofilm formation under conditions that lead to efficient sporulation, leading us to propose that there is a mechanism to inhibit biofilm formation upon activation of the sporulation pathway. To test this hypothesis, we expressed a constitutively active form of Spo0A, Sad67, that mimics high levels of Spo0A~P, in cells under biofilm formation conditions. Unlike strains expressing a wild-type version of Spo0A, strains expressing Sad67 showed a profound defect in biofilm formation and expressed a sporulation operon, *spoIIA*. Similarly, a strain lacking the Spo0A phosphatase YnzD also showed a defect in biofilm formation. The defect in biofilm formation exhibited by the *sad67* and *ynzD* mutant strains was due to a lack of repression of AbrB. AbrB protein levels were higher in a strain expressing Sad67 than in a strain expressing wild-type Spo0A. Deletion of *abrB* in either the *sad67* or the *ynzD* mutant strain also restored biofilm formation to these strains. These studies demonstrate how levels of phosphorylation of Spo0A control a molecular switch between biofilm formation and sporulation.

# S7:5

## PURIFICATION AND STRUCTURAL DETERMINATION OF SAPT, A LANTIBIOTIC-LIKE PEPTIDE INVOLVED IN AERIAL HYPHAE FORMATION IN THE STREPTOMYCETES

*S. Kodani[1], M. C. Durrant[2],* ***J. M. Willey****[1]; [1]Hofstra University, Hempstead, NY, [2]John Innes Centre, Norwich, UNITED KINGDOM*

The streptomycetes are soil dwelling gram-positive bacteria that have a complex life cycle that includes the development of an aerial mycelium and spores. In *Streptomyces ceolicolor*, the formation of aerial hyphae involves the secretion of the hydrophobic peptide, SapB, which functions to release the surface tension at the colony/air interface. We recently showed that SapB has an unusual lantibiotic-like structure, although it apparently lacks antimicrobial activity. Because it appears that aerial hyphae cannot escape the aqueous milieu of the colony without biosurfactant activity, we postulated that SapB-like molecules may be produced by other *Streptomyces* species. To explore this possibility we isolated a peptide from *S. tendae* Tü901/8c, whose exogenous application rescued developmentally blocked streptomycete mutants. Chemical analyses of the peptide including Edman degradation after chemical modification, TOF/MS-MS and NMR were performed. The peptide consists of 21 amino acids and has a

molecular weight of 2032 Da. We found that this peptide, which we call SapT, is also a lantibiotic. Unlike SapB with its two lanthionine bridges, SapT has 4 smaller loops as a result of three Me-lanthionine bridges and one lanthionine bridge. Like SapB, SapT is also very hydrophobic. Surprisingly, the *S. ceolicolor ramS* null mutant (*ramS* encodes the SapB prepeptide) treated with either SapT or SapB completed its developmental program, making not just aerial hyphae, but spores as well. This suggests that the previous assertion that SapB functions strictly as a biosurfactant may be inaccurate. Instead, SapB and SapT may have a role in signaling morphogenesis

# S7:6

## DEVELOPMENTAL BIOLOGY OF BIOFILMS

***Roberto Kolter***
*Harvard Medical School, Boston, MA*

In natural settings microbes typically reside within surface-associated multicellular communities known as biofilms. Biofilms may be as diverse as the microbes that build them; simple observation reveals a wealth of differences in their architecture, chemistry, and physiology. Nevertheless, biofilms of every sort share an important structural feature: their constituent cells are bound together by an extracellular matrix that mainly consists of macromolecules produced by the cells themselves.

Extracellular matrices have been shown to play essential roles in the developmental biology of biofilms. However, our current understanding of the biofilm matrix is rudimentary at best. The molecular composition of the matrix is likely to be complex, yet few components have been identified in even the best studied biofilms. Still less is known about the regulation of matrix components; *e.g.*, how is production of an individual component coordinated with that of the others? To address these issues we have initiated a comprehensive analysis of the extracellular matrix of biofilms produced by the spore-forming gram-positive soil bacterium *Bacillus subtilis*. This talk will present our current understanding of the developmental biology of matrix formation in this model organism.

# A1

## LOCALISATION OF THE CELL WALL SYNTHESIS MACHINERY IN BACILLUS SUBTILIS

***D. Scheffers***[1]*, J. Errington*[2]*;*
*[1]Vrije Universiteit Amsterdam, Amsterdam, THE NETHERLANDS, [2]Sir William Dunn School of Pathology, University of Oxford, Oxford, UNITED KINGDOM*

Bacterial cell shape is determined by a rigid external cell wall. In recent years, bacterial homologues of all three eukaryotic cytoskeletal structures, actin-, tubulin- and intermediate filaments have been discovered and shown to play critical roles in bacterial morphogenesis. In order to study the topological control of cell wall synthesis in bacteria, we have studied the localisation of GFP-fusions to 13 (out of a total of 16) Penicillin Binding Proteins (PBPs) in Bacillus subtilis. PBP localisation was studied both during vegetative growth and sporulation, and in strains deficient for the cytoskeletal proteins MreB, Mbl and FtsZ. This study identified three distinct localisation patterns during vegetative growth, i.e. disperse, septal and punctate. The localisation patterns were not dependent on either MreB or Mbl (Scheffers et al., 2004, Mol Microbiol 51, 749). PBP1, which localised exclusively to the cell division site, was found to be a non-essential component of the B. subtilis cell division machinery (Scheffers and Errington, 2004, J. Bacteriol 186, 5153). During sporulation, PBP2c and 2d were found to be targeted to the prespore by an, as yet unidentified, mechanism. Strikingly, the putative endopeptidase PbpX was found to spiral out to the asymmetric division site as previously described for cell division proteins FtsZ, FtsA and EzrA (Scheffers, in press). This is the first comprehensive study of PBP localisation in a single bacterium. The findings will be discussed in the view of recent findings on bacterial cell wall synthesis and PBP localisation.

# A2

## PROTEOMIC ANALYSIS OF THE MYXOCOCCUS XANTHUS EXTRACELLULAR MATRIX

***P. D. Curtis****, L. J. Shimkets;*
*University of Georgia, Athens, GA*

Cells in a biofilm are surrounded by an insoluble array of secreted macromolecules composed principally of polysaccharide and protein. An extracellular matrix (ECM) mutant of *Myxococcus xanthus* is defective in a number of essential cellular and multicellular processes, including biofilm formation, pili-based cell movement over surfaces (S motility) and fruiting body development. The protein component of the ECM is largely unknown and it is possible that proteins associated with the ECM direct these processes. The goal of this work is to identify the protein components of the ECM and determine which cellular processes they are involved in. The ECM was extracted by lysis of LS2200 (*fibA*) cells with detergent and sedimented by centrifugation. Proteins were extracted from the polysaccharide by boiling in detergent and the polysaccharide component was removed ultracentrifugation. Solublized ECM proteins were separated by 1D SDS-PAGE. Protein bands were subjected to in-gel trypsin digestion followed by peptide extraction. The extracted peptides were analyzed by MALDI-TOF Mass Spectrometry and used to identify the parent proteins using MS-FIT analysis. Several putative ECM proteins have been identified, some which have no homology to previously analyzed proteins. One putative ECM protein, encoded by ORF01085, is located in an operon with *agmX*. Transposon insertions in *agmX* have been shown to have an Adventurous motility defect. Mutations in the genes encoding ECM proteins may not only reveal new aspects of already described ECM functions, but entirely new functions for the extracellular matrix.

# A3

## DEVELOPMENTAL OSMOADAPTATION IN *STREPTOMYCES COELICOLOR*.

***L. Fernandez****;*
*University of Wales, Swansea, Swansea, UNITED KINGDOM*

Stress and development are believed to be closely linked in *Streptomyces* (Kelemen, G., 2001). The molecular basis behind this is poorly characterised. A number of biosynthetic genes are available to *S. coelicolor* which generate putative compatible solutes. In order to study the role of these genes and investigate their possible link to osmoadaptation and differentiation Transposon insertions were utilised. Insertions disrupting a putative glutamine synthetase (*SCO1613*), a probable trehalose synthase (*SCO7334*), a possible glycogen debranching enzyme (*SCO7338*), a putative aldehyde dehydrogenase (*SCO5657*) and a probable transcriptional repressor (*SCO5231*) among others were carried out. Disruption of *SCO1613* produced a bald phenotype (abnormal aerial hyphae development) in media containing osmolyte. *S. coelicolor* contains 5 possible glutamine synthetase genes whose wide variety of probable roles includes glutamate metabolism, cell wall formation and nitrogen metabolism. The specific role of *SCO1613* is still unknown. Disruption of *SCO5231* produced a bald phenotype in a glucose dependent manner. *SCO5231* has been proposed *in silico* to regulate a large number of genes (Rigali, S., 2004). Among these is *osaB*, which is part of a two-component regulatory system involved in osmoadaptation (Bishop, A., 2004). Studies with the reporter gene *luxAB* fused to the *osaB* promoter indicated that *osaB* might be repressed by *SCO5231* in a glucose dependent manner in *S. coelicolor*. Further experiments with *SCO1613* and *SCO5231* to reveal their role in this bacterium as well as studies with other potential genes involved in osmoadaptation are underway with the aim of providing a further understanding of the complex signalling pathways that occur

in *S. coelicolor*.

# A4

## STUDY OF MEMBRANE PROTEIN INTERACTIONS BY TARGETING HETEROLOGOUS PROTEINS TO THE YEAST NUCLEAR MEMBRANE

***A. Taghbalout***, *L. Rothfield;*
*University of Connecticut Health Center, Farmington, CT*

The process of division site placement in E. coli requires interaction between a membrane-bound protein, MinD, and other components of the Min system (MinC and MinE). As part of this process, MinD also self-interacts to form membrane-associated polymeric structures. While studying these interactions, we found that heterologous proteins, such as MinD, can be targeted to the yeast nuclear membrane as shown by use of fluorescently-labeled proteins. Interactions of the membrane-associated proteins can then be conveniently monitored by the yeast two-hybrid system. Targeting required the presence of a nuclear targeting sequence and a membrane-binding domain (MBD). In the case of MinD, the nuclear membrane targeting was equally effective using the intrinsic MinD MBD, or by substituting an unrelated MBD, the membrane-targeting sequence of cytochrome b5. This permitted study of chimeric proteins to define the role of membrane-association in MinD-MinD interactions and in the interactions with other proteins that lacks the MBD such as MinE and MinC.
Thus, the ability to swap membrane binding domains makes it possible to address questions about the direct role of membrane-intercalating sequences in intramembrane interactions of membrane-associated proteins, using the convenient yeast two hybrid system to monitor protein-protein interactions. A similar approach should be feasible for study of a wide range of protein-protein interactions in other systems.

# A5

## A GENE CLUSTER CONSERVED IN ACTINOBACTERIA IS INVOLVED IN COORDINATING REPRODUCTIVE GROWTH IN *STREPTOMYCES COELICOLOR*

***G. E. Jones***, *R. Del Sol, P. Dyson, S. Griffiths;*
*University of Wales, Swansea, Swansea, UNITED KINGDOM*

Recent increases in the number of fully sequenced genomes have allowed comparisons to be made between a wide variety of species. Such comparisons have led us to analyse a gene cluster conserved in all sequenced actinomycetes, using *Streptomyces coelicolor* M145 as a model organism. The cluster lies close to the origin of replication, and is thought to be involved in the timing of cell division in *S. coelicolor*. Mutants were made using transposon mediated mutagenesis in the following genes: SCO3843 (phospho - serine / threonine modulation protein), SCO3848 (ser / thr kinase), SCO3854 (CrgA), SCO3859 (putative DNA binding protein). Each individual disruption altered the parental phenotype to one of early aerial hyphal development and secondary metabolism. Localisation has been studied using his - tagged CrgA. Current work is focussing on the interactions between the gene products from members of this cluster. (J Bacteriol. 2003 Nov;185(22):6678-85.)

# A6

## SIGMA FACTOR U ACTIVITY IN STREPTOMYCES COELICOLOR

***A. Gehring***;
*Williams College, Williamstown, MA*

Unregulated activity of the *Streptomyces coelicolor* extracytoplasmic function (ECF) sigma factor, $\sigma^U$, has been shown to block differentiation in this sporulating, filamentous bacterium. A mutant in which the gene encoding *rsuA*, the presumed cognate anti-sigma factor for $\sigma^U$, is disrupted exhibits a classic "bald" phenotype characterized by failure to produce an aerial mycelium and defects in antibiotic production. Using a transcriptional fusion of the *sigU* promoter region to a *gfp* reporter, we have demonstrated that transcription of *sigU* is high in this bald mutant background (in which there is no negative regulation of $\sigma^U$ activity by RsuA) but not in wild-type or *sigU* mutant cells. Thus, *sigU* itself is a $\sigma^U$ -dependent gene. We propose a consensus sequence for $\sigma^U$ -dependent promoters based on comparison of the region upstream of *sigU* to that upstream of the *Streptomyces avermitilis* homolog and to the consensus sequences characterized for other ECF sigma factor-dependent promoters. In addition to *sigU*, six other genes in the *S. coelicolor* genome have an appropriately positioned sequence that exactly matches our proposed promoter consensus. Four of these genes encode predicted secreted proteins including two proteases. We have begun testing the validity of our proposed $\sigma^U$ -dependent promoter consensus by fusing the candidate promoters to a *gfp* reporter. The first candidate tested did indeed show high levels of transcription in the *rsuA* bald mutant but not in wild-type cells as expected for a $\sigma^U$ -dependent promoter. Work is also ongoing to characterize the presumed binding of $\sigma^U$ and RsuA and to investigate conditions that elicit $\sigma^U$ activity in a wild-type strain background.

# A7

## CONSTRUCTION OF A COSMID LIBRARY OF *STREPTOMYCES RIMOSUS* TO ISOLATE THE OXYTETRACYCLINE GENE CLUSTER

***A. Almutairi**, I. S. Hunter, P. Herron;*
*Strathclyde university, Glasgow, UNITED KINGDOM*

*Streptomyces rimosus* is a Gram-positive filamentous actinomycete which is responsible for the production of the broad-spectrum aromatic polyketide antibiotic oxytetracycline (OTC). Antibiotic production is encoded by 34kb gene cluster, which is located 600kb from the end of the unstable linear chromosome. The gene cluster and chromosome ends are subject to deletion. The *otc* genes cluster consists of a core of *otc* biosynthetic genes flanked at both ends by resistance genes. To isolate the large size *otc* cluster from *S. rimosus* a size selected cosmid library using vector pSupercos1 was constructed and around 4000 cosmid colonies were obtained. The objective of the library is to identify a cosmid clone containing the entire *otc* cluster. Southern hybridisation using chromogenic methods was applied using two distal resistance genes of the *otc* genes cluster as probes to identify the desired cosmid. The first probe was the *Eco*RI-*Hind*III 1.9kb DNA segment of the *otr*A resistance gene. The second probe was *Bam*HI-*Sac*I 1.8kb DNA segment of the *otr*B resistance gene.The cosmid containing the entire *otc* genes cluster will be used for *invitro* transposon mediated mutagenesis, to make mutations throughout the cluster, to develop a delivery system for the cosmid from *E.coli* to *S. rimosus* to study the expression of the cluster in mutant strains, and to transfer the cosmid to other *Streptomyces* species to integrate copies of the *otc* cluster and study antibiotic production in them.

# A8

## CELL DIVISION IN *BACILLUS SUBTILIS*: FTSZ AND FTSA ASSOCIATION IS Z RING INDEPENDENT AND FTSA IS REQUIRED FOR EFFICIENT MIDCELL Z RING ASSEMBLY

*S. Jensen[1], **L. S. Thompson**[2], L. J. Harry[2];*
*[1]University of Sydney, Sydney, AUSTRALIA, [2]University of Technology, Sydney, Sydney, AUSTRALIA*

Cell division is essential in the colonisation and infection process of bacteria. The earliest stage in cell division in bacteria is the polymerisation of the FtsZ protein at the division site to form the Z ring. Other division proteins are recruited to this site to complete the septation process. FtsA is a cytosolic division protein that interacts directly with FtsZ. The function of FtsA is unknown, however, it is generally believed to localize to the division site after Z ring formation and to be responsible for recruiting the later-assembling membrane-bound division proteins to this site. Here, we report the development of an *in vivo* chemical cross-linking assay to examine the association between FtsZ and FtsA in *B. subtilis* cells. Using a synchronous cell cycle system we have used this assay to show that FtsZ and FtsA interact prior to Z ring formation. Further, it is shown that in *B. subtilis* an *ftsA* deletion mutant is viable. However, the formation of Z rings is abnormal, with only 10% normal Z rings being formed. Consequently, cells of the *ftsA* deletion mutant are filamentous. It is therefore proposed that although not essential for survival, FtsA in B. *subtilis* is critical for the efficient formation of functional Z rings. This is the first report of abnormal Z ring formation resulting from the loss of a single septation protein. Our results also suggest that FtsA ensures recruitment of the membrane-bound division proteins by ensuring correct formation of the Z ring, rather than by directly recruiting these later assembling division proteins directly.

# A9

## MEMBERS OF THE SALP FAMILY PLAY A ROLE IN PEPTIDOGLYCAN ASSEMBLY AND DEGRADATION OF SPORULATION-SPECIFIC CELL DIVISION IN *S. COELICOLOR*

***E. E. Noens**[1], H. K. Koerten[2], G. P. van Wezel[1];*
*[1]University of Leiden, Leiden, THE NETHERLANDS, [2]Leiden University Medical Centre, Leiden, THE NETHERLANDS*

During its life cycle, *Streptomyces* undergoes two apparently different cell division events. Growth on solid media starts with the germination of a single spore that develops into a complex vegetative mycelium of branching hyphae that are divided into connected multinucleoid compartments by vegetative septa or cross-walls. Environmental signals result in the development of initially aseptate aerial hyphae. Developmental cell division results in the simultaneous production of up to a hundred spore septa in close harmony with chromosome segregation, resulting in chains of hydrophobic, uninucleoid spores. The molecular mechanisms underlying this process, which requires unparalleled complex coordination of cell wall synthesis, DNA segregation, and autolysis, has long been a mystery. The family of SsgA-like proteins (SALPs) is apparently unique to sporulating filamentous bacteria. Four of the SALPs are present and generally highly conserved in all streptomycetes analyzed so far, suggesting an important role in their life-cycle. Previously we have shown that *ssgA* and *ssgB* are essential for correct sporulation in *S. coelicolor*. Knock-out mutants of *ssgC-D-E-F-G* were created in *Streptomyces coelicolor* and the effect of the mutations on development and cell division were examined by a combination of electron microscopy and confocal fluorescence microscopy. These experiments revealed that

*ssgC-G* play an important role in directing septum localization, spore wall synthesis and autolytic spore separation. Confocal fluorescence microscopy was also used to study the localization of the SALPs, using fusions with Green Fluorescent Protein (GFP). Here, we discuss the role of the SALPs in the control of specific aspects of the sporulation process, and present a model to incorporate our observations and summarize their functions. Our recent data tell us that the SALPs most likely function by recruiting other proteins, such as enzymes responsible for the synthesis and degradation of the spore-wall peptidoglycan (PBPs, autolysins) (Noens et *al.*, submitted). Currently, we are searching for interaction partners for the SALPs using genomics approaches.

# A10

## DEVELOPMENTAL STAGE-SPECIFIC ASSEMBLY OF PARA AND PARB COMPLEXES IN *STREPTOMYCES COELICOLOR* HYPHAE

***D. Jakimowicz***[1]*, J. Zakrzewska-Czerwinska*[1]*, K. F. Chater*[2]*;*
*[1]Institute of Immunology and Experimental Therapy, Wroclaw, POLAND, [2]John Innes Centre, Norwich, UNITED KINGDOM*

Bacterial chromosome and plasmid segregation often involve proteins of the ParA (ATPase) and ParB (DNA binding protein) families. In the mycelial bacterium *Streptomyces coelicolor*, septation-associated chromosome segregation occurs only infrequently during vegetative growth, but it is necessary on a large scale during the formation of chains of unigenomic spores from multigenomic aerial hyphae. Disruption of either of the genes in the *parAB* operon in *S. coelicolor* leads to disturbed chromosome segregation into spores, but has no other obvious effects on colony growth. ParB binds to about 20 *parS* sites clustered around *oriC* region, forming large nucleoprotein complexes that behave differently during vegetative growth and in sporulating aerial hyphae. In vegetative hyphae, foci are irregularly spaced and smaller but often located about 1 micron from hyphal tips. In aerial hyphae in contrast, regularly spaced large foci associated with chromosome separation form immediately before sporulation septation, and they disappear after septation has been completed. The occurrence of the foci in vegetative hyphae depends on the activity of a constitutive promoter, *parAB p1*, while up-regulation of a second promoter (*parAB p2*) is a prerequisite for the assembly of foci in aerial hyphae. There was no *p2* activity in a representative set of non-sporulating (*whi*) mutants. Efficient formation of ParB partitioning complexes also depends on ParA, which has a distinctive pattern of localisation; in vegetative hyphae it is found closer to the tips than the ParB foci while in aerial hyphae it forms extended spirals along hyphal tip compartments. Localisation of ParA was independent of ParB. Our results suggest that ParB acts through spatial organisation of the *oriC* proximal part of the chromosome.

# A11

## *BORDETELLA BRONCHISEPTICA* AS MODEL ORGANISM FOR STUDYING BIOFILM IN BACTERIAL INFECTION

***Y. Irie****, M. H. Yuk;*
*University of Pennsylvania, Philadelphia, PA*

*Bordetella bronchiseptica* is a Gram-negative respiratory pathogen. A majority of its virulence factors are genetically regulated by a two-component system, BvgAS. *B. bronchiseptica* infections are chronic and often result in life-long colonization of the host. Administration of antibiotics to infected mice have been shown to be ineffective, and as *in vitro* biofilm displays enhanced antibiotics resistance, involvement of biofilm formation *in vivo* is suggested. Since common laboratory animals are natural hosts of *B. bronchiseptica*, this organism is a possible model system for studying biofilm in an actual *in vivo* host-pathogen setting. We characterized *B. bronchiseptica in vitro* biofilm formation as being predominant in the virulence intermediate phase, when adhesins are highly expressed, but toxins are down-regulated. Adhesin filamentous hemagglutinin (FHA) was found to be required for biofilm formation. In addition, expression of adenylate cyclase toxin (CyaA) was suggested to directly inhibit FHA function by direct protein-protein interaction. Upon inspecting the nasal cavity of infected animals using immunofluorescence microscopy, an *in vitro* biofilm deficient FHA mutant has shown a defect in colonization of the nasal turbinates *in vivo*. Wild type bacteria, on the other hand, colonize the epithelial surface as three-dimensional aggregated foci, strongly resembling the *in vitro* biofilm microcolonies. There are currently no biological systems for studying biofilm formation during natural bacterial infection, and *B. bronchiseptica* may represent a novel system for studying biofilm *in vivo*.

# A12

## GENE MXAN5696 ENCODES A EUKARYOTIC-LIKE PROTEIN KINASE THAT FUNCTIONS DURING EARLY DEVELOPMENT IN *MYXOCOCCUS XANTHUS*

*A. Castañeda-García, M. Martínez-Cayuela, J. Pérez,* ***J. Munoz-Dorado****;*
*Universidad de Granada, Granada, SPAIN*

*Myxococcus xanthus* is a soil-dwelling bacterium that undergoes a developmental cycle upon nutrient starvation originating fruiting bodies filled of myxospores. For the completion of the cycle, cells communicate with one another by the exchange of five different signals. Several signal transduction systems have been demonstrated to function during development, including

two-component regulatory systems and pathways where eukaryotic-like protein kinases (ePKs) are involved. The analysis of *M. xanthus* genome sequenced by TIGR/Monsanto has revealed the presence of 99 ePKs, being this number the higher found in a prokaryote and similar to that reported in eukaryotic microorganisms as *Saccharomyces cerevisiae*. In order to identify kinases that are involved in development, we have constructed several fusions between genes encoding this kind of proteins and the reporter gene *lacZ*. Determination of β-galactosidase activity in strains harbouring these fusions has allowed us the identification of several genes whose levels of expression increase during different stages of development. One of them, MXAN5696, is expressed during both vegetative growth and development, but its levels of expression increase during the first hours of development, reaching a maximum 2h after starvation on TPM medium. An in-frame deletion mutant in this gene aggregates and sporulates at a faster rate than the wild-type strain. Although the morphology of the mature fruiting bodies is identical to that of the wild-type, the number of myxospores is reduced in the mutant. These results indicates that this kinase function as a repressor of development during early development. MXAN5696 is forming part of an operon with other 10 genes, some of which encode proteins that exhibit similarities with other proteins of known functions, such as a suppressor of sigma54-dependent transcription, ABC transporters, an endonuclease and an ATPase. Results about the characterization of the operon will be discussed at the meeting.

# A13

## GLYCOSIDASES PRODUCED BY ACTINOMYCETES - TARGETING MUCIN DEGRADATION

***E. E. Gaskell***[1]*, G. Hutcheon*[2]*, G. Hobbs*[1]*;*
*[1]School of Biomolecular Sciences, Liverpool John Moores University, Byrom Street, Liverpool, UNITED KINGDOM, [2]School of Pharmacy and Chemistry, Liverpool John Moores University, Byrom Streeet, Liverpool, UNITED KINGDOM*

Mucins are high molecular weight glycoproteins ranging in size from several hundred to several thousand kDa. Their size and secondary structure is greatly influenced by their oligosaccharide side chains linked via O-glycosidic bonds to serine/threonine amino acid residues in the protein backbone. Carbohydrates account for up to 90% of the weight of these macromolecules and it is because of these highly glycosylated domains that mucins lack secondary structure. Mucins are the major glycoproteins found in the lungs of cystic fibrosis (CF) patients and are partially responsible for the highly viscous mucus produced as a consequence of this genetic disorder. This highly viscous mucus represents a major problem in drug delivery, preventing drugs from penetrating to the lower regions of the lungs. As a consequence to this, resistance to the antibiotics used has developed within the pathogenic and opportunistic microorganisms infecting the CF lung.
An array of diverse microorganisms are known to produce a range of proteases and glycosidases that may indeed exhibit affinity toward mucin like glycoproteins. Previous work identified proteolytic mucinolytic activity from various known actinomycetes and isolated strains. The current work aims to show the presence and identify the activity of the glycosidases produced by these organisms.
Preliminary results yielded a decrease in the mucinolytic activity of crude culture protein preparations, when the organisms (grown in Minimal Media (MM) containing mucin) were supplemented with glucose, compared to samples not exposed to glucose. This decrease may be the direct effect of a repressive role that simple carbon sources have on production of proteases or glycosidases.
Work undertaken aimed to compare the two specific classes of mucinolytic enzymes (proteases and glycosidases) produced under these conditions and establish their relationship and contributions to the degradation of the mucin substrate. These enzymes were studied as part of a project aimed to identify novel enzymes capable of degrading mucins. The enzymes identified and isolated will play a significant role in a drug delivery system designed with novel functional polymers to aid the delivery of an appropriate drug to patients with CF.

# A14

## DIVERSE CHANGES IN THE GLOBAL GENE EXPRESSION ARE CAUSED BY MUTATIONS IN *CDAR* AND *ABSA* IN *STREPTOMYCES COELICOLOR* A3(2)

***A. Wahab****, V. Mersinias, G. Bucca, C. P. Smith;*
*School of Biomedical and Molecular Sciences, University of Surrey, Guildford, UNITED KINGDOM*

Antibiotic production in *Streptomyces coelicolor* is a highly regulated process and this regulation occurs at different levels. We are studying one of these regulatory system, the AbsA two-component system, consisting of a sensor kinase (AbsA1) and a response regulator (AbsA2). The *absA1A2* genes are located within the *cda* cluster and have been shown to negatively regulate all four known antibiotics produced by *S. coelicolor* (Adamidis *et al.,* J. Bacteriol. 1990 **172**: 2962-2969). To further examine the role of these genes, in-frame deletion mutants were created by deleting large portions of the ORFs of both sensor kinase and response regulator genes in both the wild-type *S. coelicolor* strain and a strain lacking the *cda*R gene encoding the transcriptional activator of CDA gene cluster. Time-course transcriptome analysis of the wild-type and the *absA, cda*R and *cda*R-*absA* double mutants was performed. It was found that the expression of a large number of genes was significantly altered in both the *cda*R and *absA* mutants. However, the expression profiles of *absA* mutants are altered more dramatically. For example, under stringent ANOVA testing, expression of > 340 genes were altered in an *absA* mutant relative to the wild-type (c.a.65% down-regulated and 35% up-regulated). These include genes encoding at least 37 transcriptional regulators - some of which are up-regulated and others down-regulated in the *absA* mutant.

# A15

## MREC AND MRED CONTROL CELL WALL SYNTHESIS DURING ELONGATION IN *BACILLUS SUBTILIS*

***M. Leaver;***
*The University of Oxford, Oxford, UNITED KINGDOM*

Answering the fundamental question of how bacteria maintain their shape has been a long-standing problem in bacterial cell biology. It has recently been shown that many bacteria have actin homologues that form filamentous cables that underlie the membrane in a helical arrangement. One of these filaments in *Bacillus subtilis,* composed of the actin homolog Mbl, has been implicated in directing synthesis of the cylindrical cell wall of the rod-shaped cell. *Bacillus subtilis* has several actin homologs, one of which, *mreB,* lies in an operon with *mreC* and *mreD*. This operon is highly conserved amongst bacteria, however little is known about the function of *mreC* and *mreD*. Using conditional mutants of *mreC* and *mreD* we find that these two genes are essential for the maintenance of cell shape and viability. Cells not expressing *mreC* or *mreD* are rounded and osmotically unstable. Interestingly, we also find that the presence of magnesium ions restores cell growth, although these cells still have a shape phenotype. Electron microscopy and fluorescence microscopy reveal that cells grown with magnesium are capable of making cell wall in the septa. Genetic studies show that *mreC* and *mreD* are responsible for the coordinated cell wall synthesis that occurs in elongation and division. Localisation of GFP-MreC suggests that MreC may interact with Mbl or MreB. We hypothesize that MreC and MreD are integral membrane proteins that interact with the actin cytoskeleton on the inside of the cell in order to coordinate the cell wall synthetic machinery on the outside of the cell.

# A16

## IDENTIFICATION OF A NEW FTSZ-INTERACTING CELL DIVISION PROTEIN CONSERVED IN GRAM-POSITIVE BACTERIA

***L. W. Hamoen***[1]*, J. Meile*[2]*, W. de Jong*[1]*, P. Noirot*[2]*, J. Errington*[1]*;*
[1]*University of Oxford, Oxford, UNITED KINGDOM,* [2]*INRA, Paris, FRANCE*

Cell division in nearly all bacteria is initiated by the polymerisation of the conserved tubulin-like protein FtsZ into a ring-like structure at midcell, the so-called Z-ring. The Z-ring functions as a scaffold for the proteins that execute the synthesis of the division septum. This complex protein structure is also referred to as the divisome. The *Bacillus subtilis* divisome contains at least 9 different proteins. Aside from FtsZ, three of the proteins are cytosolic; FtsA, ZapA, and EzrA. It was recently suggested that FtsA serves as the principal membrane anchor for the Z-ring. ZapA stimulates polymerisation of FtsZ, however a deletion of *zapA* shows no apparent phenotype. An *ezrA* mutant is viable but tends to assemble multiple Z-rings both at polar and midcell positions. Genetic data suggests that the protein is a negative regulator of Z-ring formation. Four divisome proteins have their major domains outside the cell and are attached to the membrane by a single transmembranespan: Pbp2B, FtsL, DivIB and DivIC. Pbp2B is an essential septal peptidoglycan synthase. About the function of FtsL, DivIB and DivIC not much is known, but they may fulfil a regulatory role in divisome assembly and disassembly. The divisome contains one integral membrane protein, FtsW, which possibly translocates the lipid-linked precursor for the septal peptidoglycan synthesis. The divisome proteins are conserved and (functional) homologues are found in the vast majority of both Gram-positive and Gram-negative bacteria. We have now identified a new member of the divisome. This protein is encoded by the second gene in a conserved operon, and due to its role in septum development, we termed it SepB. Mutations in the *sep* locus are viable but result in elongated cells. A Yeast Two-Hybrid assay revealed a clear interaction between SepB and FtsZ, and this was corroborated by fluorescence microscopy studies. A SepB-GFP fusion accumulated at the cell division site, and this localization depended on FtsZ. It turned out that a *sepB* mutation is lethal in an *ezrA* background. In this double mutant Z-rings are still present but no division septum is formed. EM studies showed that septa in *sep* mutants are deformed, especially at the central area. SepB is conserved in Gram-positive bacteria. In *Streptococcus pneumoniae* a *sepB* mutation results in an aberrant cell division as well. We conclude that SepB is a new member of the Gram-positive divisome, and propose that it functions in the late steps of septum synthesis.

# A17

## BIOSYNTHESIS OF THE ADHESIVE HOLDFAST POLYSACCHARIDE IN *CAULOBACTER CRESCENTUS*

***E. Toh****, Y. V. Brun;*
*Indiana University Bloomington, Bloomington, IN*

*Caulobacter crescentus* is an aquatic bacterium that leads a dimorphic lifestyle, generating a sessile stalked cell, and a motile swarmer cell. Stable attachment of *Caulobacter* stalked cells to surfaces requires an adhesive polar organelle, the holdfast. The holdfast is composed in part of oligomers of the sugar N-acetylglucosamine (NAG) which are localized to the tip of the stalk. A previous genetic screen identified adhesion defective mutants which had transposon insertions in a gene cluster involved in polysaccharide export, *hfsDABC*. Adjacent to *hfsDABC*, we identified another four genes named *hfsEFGH,* which have sequence similarity to polysaccharide biosynthesis genes. HfsE is homologous to UDP-sugar lipid carrier transferases that catalyze the transfer of NAG from

UDP-NAG to undecaprenol phosphate carrier. HfsG is similar to glycosyltransferases that transfer the sugar from UDP-glucose and UDP-NAG to the growing repeat unit. HfsH resembles polysaccharide deacetylases. HfsF is similar to *E.coli* Wzx, a putative flippase, which catalyzes the translocation of undecaprenol-phosphate-linked polysaccharide across the inner membrane. HfsC and its paralog HfsI share homology to *Salmonella* Wzy, a putative polymerase of individual repeat polymer units. Clean in-frame deletion mutants of *hfsE, hfsF, hfsG*, and *hfsH,* all exhibit decreased adherence to a polystyrene surface based on a crystal violet assay, namely 15%, 38%, 8%, and 5%, respectively as compared to wild-type CB15 cells (100%). Mutants with clean deletions of *hfsC* or *hfsI* exhibited no deficiency in surface adhesion, but a double *hfsC hfsI* mutant was severely deficient with 2% binding as compared to wild-type. *hfsE, hfsF, hfsG, hfsH*, and the double *hfsC hfsI* mutants also have significantly lower levels of holdfast NAG, as assayed by labeling with wheat-germ agglutinin (FITC-WGA), which binds specifically to NAG. Based on these data and sequence similarity, we propose that the biosynthesis of the holdfast polysaccharide follows a Wzx/Wzy-dependent translocation pathway, where HfsE transfers the nucleotide diphosphosugar precursors to a carrier lipid, and sequential activities of glycosyltransferase enzymes at the cytoplasmic face of the inner membrane, like HfsG help assemble the individual repeat units. The repeat units would then be exported across the inner membrane, by a process involving the flippase HfsF, to the periplasm where they are polymerized by HfsC and HfsI.

# A18

## RINGS AND SPIRALS OF THE CELL DIVISION PROTEIN, FTSZ: NEW LESSONS FROM AN OLD MUTANT

***E. J. Harry****, L. G. Monahan, K. A. Michie;*
*University of Technology, Sydney, Sydney, AUSTRALIA*

The earliest stage in bacterial cell division is the formation of a ring, composed of the tubulin-like protein, FtsZ, at the division site. The Z ring acts as a scaffold for the assembly of the division apparatus and contracts at the leading edge of the developing septum during cytokinesis. Tight spatial and temporal regulation of Z ring formation is required to ensure that division occurs precisely at midcell between two replicated chromosomes.

The self polymerization of FtsZ has been well characterized *in vitro*. However, the structure of the Z ring, and the mechanism and regulation of ring formation *in vivo* remain unresolved. More recent data has revealed an intrinsic ability of FtsZ to assemble into a dynamic spiral during normal growth and development. We have identified dynamic spiral-like FtsZ polymers in *B. subtilis* during vegetative growth and spore outgrowth. These spirals are able to form along the length of the cell and appear to precede Z ring formation at the division site at midcell.

Using molecular and cell biology approaches, we have identified the defect of the only reported *ftsZ* mutant *(ts1)* of *Bacillus subtilis.* At the non-permissive temperature, the mutant protein, FtsZ(Ts1), shows an intriguing cellular localization pattern. It assembles into short spiral-like structures between chromosomes. When shifted back down to the permissive temperature, functional Z rings form by reorganization of these Z spirals and division resumes. Our observations support a model for wildtype cells in which Z ring formation at the division site arises from reorganization of a cytoskeletal spiral form of FtsZ and suggest that the FtsZ(Ts1) protein is captured as a spiral-forming intermediate that is unable to complete this reorganization step. Unlike the FtsZ spirals that form in wildtype cells, the FtsZ(Ts1) spirals form only in nucleoid-free areas. The *ts1* mutant is likely to be very valuable in revealing how FtsZ assembles into a ring and how this occurs precisely at the division site.

# A19

## COORDINATION OF CHROMOSOME SEGREGATION AND HYPHAL GROWTH IN *STREPTOMYCES COELICOLOR*

***P. R. Herron***[1]*, E. J. Tilley*[1]*, T. Huang*[2]*, C. Chen*[2]*;*
[1]*University of Strathclyde, Glasgow, UNITED KINGDOM,*
[2]*National Yang Ming University, T'aipei, TAIWAN REPUBLIC OF CHINA*

Recent evidence indicates that an unknown primary cellular positioning mechanism exists in bacteria, at least in *Escherichia coli*, that coordinates chromosome segregation with cell division. This model proposes that, before a mother cell divides into two daughter cells, a succession of events must take place at mid-cell in a precise order. Following growth of the mother cell to a critical cell length, the primary cellular positioning mechanism sequentially places the chromosome's origin of replication, the replication factory, a ring of the cytokinetic protein FtsZ and the septum at mid-cell. *Streptomycetes* differ from other bacteria in that their growth appears to be monodirectional, although, at present, it is unclear whether growth occurs by intercalary assimilation of peptidoglycan or it is incorporated at the hyphal tip. In *E. coli*, a primary cellular positioning mechanism and bidirectional chromosome segregation can be easily accommodated within the former mode of peptidoglycan incorporation and, as a result, the mechanism for cell division by binary fission. Unless streptomycetes possess a novel bacterial system for chromosome positioning, segregation and division site selection it is not possible to reconcile bidirectional chromosome segregation with monodirectional hyphal extension. In order to resolve this apparent paradox we have analysed mutants of the *S. coelicolor* homologue of the *Escherichia coli* cell division gene, *ftsK* using fluorescence *in situ* hybridization (FISH) and measurement of prespore interseptal distances.

# A20

## EVOLUTION FROM OBLIGATE CHEATING TO SOCIAL INDEPENDENCE IN *M. XANTHUS*: A MOLECULAR GLIMPSE

***S. V. Kadam***[1], *J. Jakobsen*[2], *L. Sogaard-Andersen*[2], *G. J. Velicer*[1]; [1]*Max-planck Institute for Developmental Biology, Tuebingen, GERMANY,* [2]*Max-planck Institute for terrestrial Microbiology, Marburg, GERMANY*

*Myxococcus xanthus* is a social, Gram-negative, soil dwelling bacterium. Its three major social traits are cooperative motility, cooperative predation and cooperative multicellular development. These genetically determined social traits of *M. xanthus* can evolve rapidly. In an earlier study, 'cheating' genotypes of *M. xanthus* evolved in an environment that selected for losses of developmental proficiency. One such cheater strain, here termed Obligate Cheater (OC), is completely developmental defective in pure culture but sporulates more efficiently than the wild type (WT) when present as a minority in mixtures with WT. In a following competition experiment, OC was mixed (1%) with WT (99%) and allowed to compete through several sequential rounds altering growth and development. After the fourth round of development, a new genotype 'SI' (Socially Independent) derived from OC reached near fixation in the population. Although OC produces no spores in isolation, its evolutionary descendant regained developmental proficiency, sporulates seven-fold more efficiently than WT in pure culture and has a different fruiting phenotype. In mixed competitions with WT, SI rapidly rises from low frequency to dominance. The evolutionary transition from OC to SI represents an 'escape' from obligate social dependence to independent social dominance. Using real time PCR, we have found differences in the expression profiles of six developmentally regulated genes (*sdeK*, *csgA*, *fruA*, *Ω 4521*, *dev*R and *exo*) across WT, OC and SI. We postulate that the dramatic phenotypic transition from OC to SI is due to a single regulatory mutation with numerous pleiotropic effects.

# A21

## ROLE OF A MEMBRANE PROTEIN INSERTASE IN THE COMPARTMENT-SPECIFIC ACTIVATION OF SIGG DURING SPORULATION IN BACILLUS SUBTILIS

***M. Serrano***[1], *C. P. Moran Jr*[2], *A. O. Henriques*[1]; [1]*ITQB, Oeiras, PORTUGAL,* [2]*Emory University School of Medicine, Atlanta, GA*

Following asymmetric division, $\sigma^F$ in the prespore and $\sigma^E$ in the mother cell control early stages of endospore differentiation in *Bacillus subtilis*, and are replaced at later stages by $\sigma^G$ and $\sigma^K$, respectively. Transcription of *spoIIIG* (encoding $\sigma^G$) is driven by $\sigma^F$ and hence is confined to the prespore. However, following synthesis $\sigma^G$ is kept in an inactive state. Activation of $\sigma^G$ is coupled to the completion of engulfment of the prespore by the mother cell, when the prespore is fully surrounded by the two membranes that derive from the asymmetric septum. Activation of $\sigma^G$ also requires expression of the *spoIIIA* and *spoIIIJ* loci. *spoIIIJ* is expressed during vegetative growth, and encodes a membrane protein insertase of the YidC/OxaP1 family. SpoIIIJ is not normally required for viability during the exponential growth phase because of expression of a SpoIIIJ paralogue, the YqjG protein, which does not function efficiently during sporulation, and may not be able to properly handle sporulation-specific substrates. SpoIIIJ accumulates in both the prespore and the mother cell but expression of *spoIIIJ* in the prespore is sufficient for $\sigma^G$ activation and wild type levels of sporulation. This suggests that SpoIIIJ is involved in the insertion in the prespore inner membrane of a protein required for activation of $\sigma^G$. The products of the $\sigma^E$-controlled, mother cell-specific *spoIIIA*-operon are candidates for SpoIIIJ substrates. The *spoIIIA* operon encodes 8 membrane-associated proteins, all of which are required for $\sigma^G$ activation. Moreover, we found that expression of the *spoIIIAE*, *spoIIIAF*, and *spoIIIAH* cistrons of the *spoIIIA* operon in the prespore compartment permits $\sigma^G$ activation and efficient sporulation. We infer that these proteins somehow reach and function in the prespore inner membrane, or that regardless of their compartment of synthesis, they can reach addresses in either or both of the prespore membranes. Furthermore, we also found that expression of *spoIIIAE* (but not of *spoIIIAF*, or *spoIIIAH*) during growth results in a lytic phenotype in the absence of a functional *spoIIIJ* locus. We propose that this synthetic lethal effect reveals a functional interaction between *spoIIIJ* and *spoIIIAE*, and suggest that SpoIIIJ acts during sporulation by facilitating the insertion of SpoIIIAE into the prespore membranes.

# A22

## EARLY EXPONENTIAL PHASE-ASSOCIATED PROTEIN SER/THR KINASE WITH WD-40 REPEATS FROM THERMOPHILIC ACTINOMYCETE *THERMOMONOSPORA CURVATA*

***K. Petrickova***, *J. Hasek, O. Benada, M. Petricek; Institute of Microbiology AS CR, Prague, CZECH REPUBLIC*

The *pknA* gene from *T. curvata* encodes a hybrid protein with the N-terminal eukaryotic-type protein Ser/Thr kinase domain and the C-terminal WD-40 repeat module. The WD-40 domain is a propeller-forming domain involved in protein-protein interactions. It is present in many eukaryotic proteins of various functions, but is very scarcely found in prokaryotes

and nothing is known about the function of these proteins in actinomycetes. Also, connection of the WD-40 domain with an enzymatic domain in a single polypeptide is quite atypical. The gene is transiently expressed during the growth of *T. curvata* in the liquid culture. PkwA concentration peaks in the beginning of the exponential growth and the protein disappears upon entering the mid-exponential phase. It is present in the cells at very low concentrations and forms *in vivo* high-molecular-weight complexes. The protein has been shown to be associated with the cell membrane. It can be visualized in the cytoplasmic membrane of *T. curvata* germinating spores by immunofluorescence microscopy using anti-PkwA monoclonal antibody. Genes surrounding *pkwA* in the chromosome putatively encode subunits of DNA polymerase III and DNA helicase, what may connect PkwA to the process of replication.

# A23

## CO-AGGREGATION OF THERMOPHILIC ISOLATES

***G. Lin***[1], *L. Lin*[1], *S. Chung*[1], *M. Chen*[2], *S. Tsay*[2]; [1]*Tzu-Chi University, Hualien, TAIWAN REPUBLIC OF CHINA,* [2]*National Taiwan University, Taipei, TAIWAN REPUBLIC OF CHINA*

We have isolated at least 3,000 of thermophilic isolates from geothermal areas of Taiwan. There are 12 genus including 33 species of bacteria in the storage library. *Meiothermus taiwanensis, Pseudoxanthomonos tainwanensis,* and *Ruberbacter taiwanensis* are new species which were studied in detail and published among those isolates. Biofilms formation is one of the characteristic which was surveyed of those isolates. According to the data of surveillance, we found 48% of those isolates could form biofilms after screened for 300 isolates randomly. Biofilms architectures and formation ability of different isolates were different among those isolates. Different carbon sources also demonstrated different importance for biofilms formation. Co-aggregation was a general phenomenon while culturing biofilms forming and non-forming strains together. Cell-free cultured supernatant of biofilms forming strain (*Thermus thermophilus* HB8) provided a heat liable substance for biofilms formation of a non-forming strain (*Deionococcus* sp. NTU553). C8-reverse phase HPLC column was applied for the quorum sensing molecules purification. Results revealed that a small molecule with m/z value of 173 in the cell-free supernatants might help *Deionococcus* sp. NTU553 for biofilms formation. Further studies of the production and regulation of those small molecules will be the major works in the future.

# A24

## TIME-LAPSE ANALYSIS OF TERMINAL ORGANELLE FORMATION AND CELL DIVISION IN MYCOPLASMA PNEUMONIAE BY MICROCINEMATOGRAPHY AND FLUORESCENT PROTEIN TAGGING

***B. M. Hasselbring***, *R. W. Krause, D. C. Krause; University of Georgia, Athens, GA.*

Mycoplasma pneumoniae is the leading cause of pneumonia in older children and young adults. With a cell volume 1/10 that of Escherichia coli and a minimal genome, this cell wall-less prokaryote is also one of simplest known self-replicating life forms. But despite its otherwise minimal nature, M. pneumoniae possesses an ultrastructurally complex, polar, membrane-bound extension of the cell body. This terminal structure functions both in mycoplasma adherence to host tissue and in gliding motility. Moreover, formation of a duplicate terminal organelle adjacent to the first and subsequent migration of one terminal structure to the opposite pole is thought to precede cell division. Given the importance of M. pneumoniae as a human pathogen, and the biologically unique features of fundamentally important cellular processes in this species, we utilized fluorescent protein tagging of a terminal organelle protein to monitor the duplication and movement of the terminal organelle in growing cultures. The adhesin P30 is an integral membrane protein that localizes solely to the terminal organelle. A P30-EYFP fusion was introduced into a P30 (-) cytadherence mutant by transposon delivery, restoring a wild-type phenotype to transformants, and thereby indicating that the fusion protein was functional. Mycoplasmas cultured in chamber slides at 37 C were monitored by phase and fluorescence digital microscopy, enabling localization and observation of fluorescent P30-EYFP foci over time. Fields were monitored over intervals of 2, 8, or 16 hr, with phase images recorded a 2, 5, or 10 min and fluorescent images recorded at 6, 30, or 60 min, respectively. Non-dividing cells generally had a P30-EYFP focus at one cell pole and were actively engaged in periods of cellular gliding. Cell division began with cessation of gliding, with formation of a second fluorescent focus adjacent to the first within 1 hr. Nascent fluorescent foci were subsequently observed to migrate towards the opposite cell pole, but often before that migration was complete up to two additional fluorescent foci formed. Over a period of 4 to 8 hr from onset of the first duplication event, a daughter cell having the original fluorescent focus could be seen gliding away from its siblings. These observations suggest that cell division in M. pneumoniae may be more complex and less ordered than previously thought. Finally, we also examined mixed cultures of M. pneumoniae ± the P30-EYFP fusion over several days in chamber slides to study microcolony development. Fluorescent and nonfluorescent mycoplasmas were widely scattered and evenly dispersed, yet microcolonies clearly

consisted predominantly of either fluorescent or non-fluorescent cells, suggesting that microcolonies result from siblings that remain nearby after multiple rounds of cell division rather than by convergence of gliding mycoplasmas.

# A25

## THE CHROMOSOME SEGREGATION PROTEIN SPO0J SPREADS ALONG DNA FROM A *PARS* NUCLEATION SITE

***H. Murray***[1], *H. Ferreira*[2], *J. Errington*[1];
[1]*University of Oxford, Oxford, UNITED KINGDOM,* [2]*Centro de Biologia Molecular Estrutural, Campinas, BRAZIL*

Regulation of chromosome inheritance is essential to ensure proper transmission of genetic information. In *Bacillus subtilis* the Spo0J protein is required for accurate chromosome segregation and it regulates the developmental switch from vegetative growth to sporulation. Spo0J is a DNA-binding protein that forms discrete foci associated with the *oriC* region of the chromosome throughout the cell cycle. In this report we characterize the DNA binding properties of Spo0J. In vivo we find Spo0J associates with the DNA flanking its binding site, *parS*, suggesting that the protein directly or indirectly contacts DNA several kilobases away from its specific binding site. Using purified components we confirm that Spo0J specifically binds to a *parS* site, and we provide evidence supporting a model where the initial *parS*:Spo0J complex recruits further Spo0J molecules that sequentially bind and spread along the substrate. The results suggest Spo0J coats a substantial region of DNA surrounding *parS* nucleation sites, thereby providing a large structure near *oriC* able to interact with other members of the chromosome segregation and/or regulatory machinery.

# A26

## NEW GENES INVOLVED IN SPORULATION IN *STREPTOMYCES COELICOLOR* A3(2)

***N. Ausmees***[1], *M. Elliot*[2], *H. Wahlstedt*[1], *M. J. Buttner*[3], *K. Flärdh*[4];
[1]*Uppsala University, Uppsala, SWEDEN,* [2]*McMaster University, Hamilton, ON, CANADA,* [3]*John Innes Centre, Norwich, UNITED KINGDOM,* [4]*Lund University, Lund, SWEDEN*

Sporulation in *Streptomyces coelicolor* involves several consecutive steps of cellular differentiation: a germinating spore gives rise to a vegetative mycelium, which then forms white aerial hyphae, which in their turn differentiate into spore chains, and finally into mature dark grey spores. Such extensive morphological changes are likely to be accompanied by substantial changes in gene expression. We have used a microarray-based approach to reveal candidate genes for further functional investigation for a role in development. Gene expression profiles of a wildtype *S. coelicolor* strain and mutant strains, which are blocked in development after raising white aerial hyphae (*whiG*, *whiI*, *whiH, whiA*, *whiB*) were obtained in a developmental timecourse experiment. Different criteria can be used to delimit a set of candidate genes for a role in development. First, we asked for genes whose expression increased during development in the wildtype strain, but failed to increase in at least one of the *whi* mutant strains. Ten genes/operons with expression profiles satisfying the above criteria were then subjected to systematic functional studies. For example, a putative operon of two genes, *SCO1415-1416*, was upregulated after 42 hours in the wildtype strain, but not in any of the *whi* mutant strains. S1 nuclease protection assays confirmed the expression pattern, and revealed a transcriptional start site at around 35 bp upstream the *SCO1415* start codon. The putative promoter region did not contain known regulatory motifs, indicating an indirect dependence of *SCO1415-1416* expression on the sporulation sigma factors encoded by *whiG* and *sigF*. However, since several cell types are present at any time in a developing *S. coelicolor* culture, RNA-based gene expression studies yield poor temporal and spatial resolution. Fluorescence microscopy with a *SCO1415-egfp* fusion strain clearly showed that the SCO1415 protein accumulated specifically in septating aerial hyphae and in young spores. Furthermore, a *SCO1415-1416* deletion strain exhibited markedly reduced pigmentation of the spores, indicating a role for SCO1415-1416 in spore maturation. Interestingly, the SCO1415 protein belongs to a family of small membrane proteins conserved in other *Streptomyces* species, and the SCO1416 protein is similar to FtsK/SpoIIIE-like ATPases. Thus, our approach has a potential to reveal novel developmental genes and shed new light on molecular mechanisms involved in differentiation and development.

# A27

## CELL SHAPE DETERMINATION IN *CAULOBACTER CRESCENTUS*

***M. A. Aaron***, *C. Jacobs-Wagner;*
*Yale University, New Haven, CT*

From prokaryotes to higher organisms, the determination and maintenance of cell shape are key cellular processes. It has been well established that the peptidoglycan cell wall of bacteria serves as an exoskeleton and maintains cell shape. Recently it has been shown that bacteria, like eukaryotes, also contain cytoskeletal elements that play a role in determining cell shape. *Caulobacter crescentus* is a crescent-shaped rod. MreB is an actin-like protein that is important for creating the rod-shape of the cell. *C. crescentus* also possess an intermediate filament-like protein, crescentin, which is required for curving the rod to create the crescent shape of *C. crescentus*. Crescentin forms a filamentous structure that localizes near the membrane at the inner curvature of the cell; however, the exact mechanism by which crescentin generates a crescent-shaped cell remains to be determined. Using a visual genetic screen, a *whqL* mutant was identified which has a straight-rod morphology. In a *whqL* mutant, the crescentin structure has an

abnormal pattern of localization and seems unable to make a productive interaction with the membrane. Interestingly, O-antigen is also altered in a *wbqL* mutant. Epistasis experiments indicate that the altered O-antigen itself causes the loss of cell curvature.

# A28

## DIFFERENTIATION PROCESSES IN THE BACTERIUM SORANGIUM CELLULOSUM

***S. D. Doß****, T. Knauber, A. Treuner-Lange;*
*Justus-Liebig-University of Giessen, Giessen, GERMANY*

Besides Streptomyces and Bacillaceae the myxobacteria are also important producers of secondary metabolites and most of these compounds appear to be developed to inhibit eukaryotic competitors in the soil habitat. In the group of the myxobacteria, Sorangium strains are the most important secondary metabolite producer and strain So ce56 was chosen for a genome project. In So ce56 two metabolites, Chivosazole A and Etnangien could already be identified. Both substances are polyketides, which are a large family of natural products found in bacteria, fungi and plants. Many clinically important drugs such as tetracycline are polyketides. Streptomyces, Bacillaceae and the myxobacteria have in common that they are able to undergo differentiation processes. In Bacillus subtilis a production of more or a longer production of secondary metabolites could be observed if early differentiation genes are knocked out. This led to the question, if blocking of the developmental pathway in Sorangium cellulosum leads to an overproduction of secondary metabolites or to a prolonged synthesis phase. Furthermore there is nearly nothing known about the differentiation process in Sorangium cellulosum, so we are also interested to investigate the differentiation process in general. Based on the hypothesis described before and the assumption that the differentiation process in Sorangium cellulosum is similar regulated as in Myxococcus xanthus, my first goal was to construct early developmental mutants in Sorangium cellulosum and to analyze them in regard to their differentiation processes. So far myxobacterial development has only been investigated in M. xanthus and one of the first genes crucial for the myxobacterial development in M. xanthus is a Lon-protease. Therefore we started to look for Lon-encoding genes in the genome of S. cellulosum and surprisingly found five different Lon-encoding genes. In contrast to S. cellulosum, M. xanthus comprises only two Lon-proteases. We started to analyze the expression of these genes by Realtime RT PCR and to construct the corresponding mutants. We will report our results concerning these genes and the first mutants in regard to their morphological and physiological differentiation. Analysis of the genome of S. cellulosum indicates that the regulation of the differentiation in S. cellulosum differs from M. xanthus. Therefore a random mutagenesis was performed and the resulting mutants were analyzed concerning their morphological and physiological differentiation. We are currently investigating the affected genes in these mutants.

# A29

## A MECHANISM FOR ESTABLISHING PROKARYOTIC CELL POLARITY

***P. L. Obuchowski****, C. Jacobs-Wagner;*
*Yale University, New Haven, CT*

How cell polarity is established is one of the fundamental questions underlying the field of cell biology. Cell polarity, marked by the uneven distribution of proteins and organelles, leads to asymmetric cell division, in which the resulting progeny inherit a different developmental fate. The aquatic bacterium *Caulobacter crescentus* provides an excellent model system for understanding the basis of prokaryotic cell polarity. Its cell cycle is coupled to polar morphogenesis, exemplified by the growth of polar organelles, including the stalk, pili, and single flagellum. In eukaryotic systems, many of the factors governing cellular asymmetry encode large amounts of the coiled coil motif in their primary amino acid sequence. A hallmark of such proteins is their ability to localize to specific subcellular addresses. In order to identify potential proteins involved in cell polarity in *Caulobacter*, twelve uncharacterized coiled coil-rich proteins were tagged with GFP in order to see if they localized to the poles of the cell. Of these, several showed distinct patterns of subcellular distribution. One of these proteins, here called PflI, has a novel pattern of cell cycle-dependent polar localization and is necessary for the formation of a single polar flagellum. Interestingly, species possessing a homolog of PflI have a unipolar flagellum during at least one part of their development, and closely related species with multiple flagella do not have a recognizable PflI homolog. We propose a model in which PflI serves as a molecular scaffold to ensure that there will be one and only one flagellum formed during the cell cycle and that this flagellum will be synthesized at the appropriate subcellular position.

# A30

## FUNCTION OF THE *BACILLUS SUBTILIS* CELL DIVISION PROTEIN FTSL

***M. Bramkamp****, J. Errington;*
*Universtity of Oxford, Oxford, UNITED KINGDOM*

Cell division (cytokinesis) is one of the pivotal biological processes in all cells. In prokaryotes the site of cell division is usually marked by the assembly of a so called Z-ring which is composed of the bacterial tubulin homologue FtsZ. Proteins involved in division localise in a defined hierarchical order to the contractile Z-ring, forming a multisubunit complex. Among other proteins, the division complex of *Bacillus subtilis* contains four bitopic proteins, FtsL, DivIC, DivIB and

PBP2B, but only PBP2B has a deduced biochemical function, in the late steps of peptidoglycan synthesis. FtsL, DivIC and DivIB (the latter are homologous to the *Escherichia coli* FtsB and FtsQ) are targeted to the division site. The secondary structure of FtsL and DivIC is mainly composed of an external C-terminal domain, with a predicted coiled-coil structure, in which the characteristic heptad motif of a leucine zipper protein is present. Depletion of FtsL has no effect on Z-ring formation but prevents the localization of DivIC, DivIB and PBP2B and leads to filamentation of the cells. Immunoblot analysis made it clear that FtsL is intrinsically instable. However, no distinct function could be attributed to any of the FtsL (or DivIC) domains despite the requirement for correct targeting to the septal site. Here we show that the cytoplasmic domain of FtsL contains the signal for rapid degradation and seems to have an important role in FtsL function during sporulation. N-terminal truncations of FtsL lead to increased stability of the protein, but do not affect correct localization of FtsL to the septum, though they have a profound impact on sporulation. Interestingly, we were able to show that a putative membrane bound zinc metalloprotease (YluC) seems to be involved in FtsL degradation. In a *yluC* knockout strain both FtsL and DivIC are more stable. The sporulation efficiency of an *yluC* knockout strain was decreased by 95 % compared with the wild-type strain. Therefore, we assume that FtsL is a substrate of regulated intramembrane proteolysis, thereby regulating disassembly of the division complex. Our results support a regulatory role for FtsL in the division complex.

# A31

## A NOVEL SURVEILLANCE MECHANISM FOR GENOMIC INTEGRITY DURING SPORULATION IN *BACILLUS SUBTILIS*

***Y. Oppenheimer-Shaanan**, M. Bejerano-Sagie, I. Berlatzky, S. Ben-Yehuda;*
*The Hebrew University of Jerusalem, Jerusalem, ISRAEL*

Genome stability is of primary importance for the survival and proper functioning of all organisms. In response to DNA damage or replication block, cells activate checkpoint signaling cascades to control cell cycle progression and elicit DNA repair in order to maintain genomic integrity. Although DNA damage checkpoint pathways are extensively studied in eukaryotic cells, such mechanisms are less characterized in prokaryotes. We describe the identification of a novel protein DisA, (for DNA integrity surveillance), which is expressed early during the developmental process of sporulation in the bacterium *Bacillus subtilis*. We found that DisA is required for a temporary arrest during initiation of sporulation in response to DNA damage. Fluorescence microscopy analysis revealed that cells bearing a *disA*⊥ allele fail to delay sporulation in the presence of chromosomal lesions caused by DNA damaging agents, such as nalidixic acid and mitomycin C, and upon induction of a specific double strand break. As a consequence, spores viability is significantly decreased in the mutant compared to wild-type cells. However, no major difference in cells viability is observed between the wild-type and *disA*⊥ cells when treated with DNA damaging agents during vegetative growth. Our data suggest that DisA plays a key role in the activation of a developmental checkpoint pathway. We propose that DisA acts by transducing a signal that causes a delay in entering to sporulation after sensing of DNA damage.

# A32

## REMOTE SENSES OF A SOCIAL GLIDING BACTERIUM

***W. P. Black**, Q. Xu, Z. Yang;*
*Virginia Polytechnic and State University, Blacksburg, VA*

*Myxococcus xanthus* utilizes surface gliding motility to aggregate during the development of multicellular fruiting bodies. At least two extracellular surface structures, type IV pili (TFP) and extracellular fibril polysaccharide or exopolysaccharide (EPS), are essential for the social (S) component of *M. xanthus* gliding motility. Retraction of TFP is proposed to power S motility and EPS from neighboring cells is suggested to provide an anchor and trigger for TFP retraction. The production of EPS is regulated in part by the Dif (defective in fruiting/fibrils) chemotaxis-like pathway; however, the input signal for the Dif pathway with regard to EPS production remains to be discovered. In this study, we demonstrate that TFP function upstream of the Dif proteins using genetic methods combined with quantitative and qualitative analysis of EPS production. We confirmed the requirement of TFP for EPS production using various classes of TFP mutants. Construction and examination of double and triple mutants of *dif* and *pil* indicated that mutations in *dif* are epistatic to those in *pil*. Furthermore, mixing experiments between various TFP and *dif* mutants suggest that TFP, instead of acting as signals, may constitute the sensor or part of the sensor responsible for mediating input signals into the Dif pathway. Our results suggest that there is a regulatory loop that couples EPS production to TFP function and vice versa. We propose that TFP, as extracellular and polarly localized protein filaments, act in essence as remote sensors to detect other cells from a distance.

# A33

## THE HYPHOMONAS NEPTUNIUM GENOME PROJECT

***J. H. Badger**[1], T. R. Hoover[2], Y. V. Brun[3], R. M. Weiner[4], M. T. Laub[5], N. L. Ward[1];*
*[1]The Institute for Genomic Research, Rockville, MD, [2]University of Georgia, Athens, GA, [3]Indiana University, Bloomington, IN, [4]University of Maryland, College Park, MD, [5]Harvard University, Cambridge, MA*

The complete genome of the marine dimorphic prosthecate

alphaproteobacterium *Hyphomonas neptunium* ATCC 15444 was sequenced by the whole genome shotgun method. Its genome is comprised of a single circular chromosome of 3,705,021 base pairs. 3,527 protein coding genes were predicted. Among these are many homologs to cell-cycle genes from *C. crescentus*, a previously sequenced alphaproteobacterium which shares a similar life-cycle to H. neptunium. Although the classification of the alphaproteobacteria is in flux (Badger, Eisen, and Ward, 2005), it is clear that *Caulobacter* and *Hyphomonas* share a relatively recent ancestor and a biphasic life cycle. The genome of *Hyphomonas neptunium* ATCC 15444 is also notable for containing many genes involved in the breakdown of aromatic compounds. These are thought to be present in the harbor environment from which the strain was isolated and may point to the possible use of the organism as a bioremediation tool.

# A34

## YHXB: AN INHIBITOR OF FTSZ RING ASSEMBLY IN BACILLUS SUBTILIS

***A. H. Lee**, R. B. Weart, P. A. Levin;*
*Washington University in Saint Louis, Saint Louis, MO*

During the bacterial cell cycle, the prokaryotic tubulin homolog, FtsZ, polymerizes into a ring structure (the Z ring) that establishes the location of the division site. The Z ring is responsible for recruiting other cell division proteins to the nascent septal site and for promoting assembly of the division machinery. As a means of identifying factors that modulate Z-ring formation in *Bacillus subtilis*, we employed a transposon mutagenesis strategy to identify inhibitors of FtsZ assembly. One gene identified in this screen encodes YhxB, a putative phosphomannomutase (PMM) or phosphoglucomutase (PGM). The loss of *yhxB* allows cells carrying a heat-sensitive allele of *ftsZ* (*ftsZts*) to form Z rings at the non-permissive temperatures of 45°C. Additionally, a *yhxB* null mutation suppresses the cell division defect and lethality associated with 15-fold overexpression of the division inhibitor MinCD. Significantly, although FtsZ localization is not perturbed, *yhxB* null mutants are 20% shorter than wild type cells, suggesting they may be defective in coupling cell growth to division. Together, these genetic studies establish YhxB as an inhibitor of FtsZ ring assembly. Characteristic of PMM/PGM proteins, YhxB has a conserved catalytic serine (S146) residue that is required in sugar metabolism. We find that mutations in this conserved serine (S146A and S146D) behave identically to *yhxB* null mutants with regard to FtsZ assembly. Future studies will be directed at testing if YhxB inhibits FtsZ through direct interactions or if YhxB serves as an indirect link between metabolism and cell division. Additionally, YhxB has been shown to be required for biofilm formation in *B.subtilis* [Branda et al., J Bacteriol 186(12):3970-9; Lazarevic et al., Appl Environ Microbiol 71(1):39-45]. Thus, it is intriguing to speculate that we have uncovered a potential regulatory pathway controlling changes in cell cycle status between actively dividing planktonic cells and those in a mature biofilm.

# A35

## REGULATION OF THE *CAULOBACTER* CELL CYCLE IS DEPENDENT ON THE PROPER SPATIAL AND TEMPORAL LOCALIZATION OF THE CELL CYCLE KINASE CCKA

***P. S. Angelasro**, C. Jacobs-Wagner;*
*Yale University, New Haven, CT*

Events controlling the cell cycle and polar morphogenesis of *Caulobacter crescentus* are performed by members of the two-component signal transduction protein family. CckA, an essential hybrid histidine kinase, is responsible for the phosphorylation of the master cell cycle transcriptional regulator CtrA. Similar to other components of this pathway, CckA localizes to the poles in a cell-cycle dependent manner. Internal deletions of CckA fused to the monomeric green fluorescent protein (CckA-mGFP) have revealed two regions necessary for proper spatial and temporal localization of CckA. These two regions are 1) a small loop in the ATP binding domain and 2) a putative PAS motif in the sensor domain. Mutants missing either of these regions support cell viability in the absence of wild-type CckA; however, the mutants have defects in cell growth, division and shape. The results presented suggest that appropriate polar localization of CckA is necessary for faithful cell cycle progression and cell morphology.

# A36

## IDENTIFYING NEW DEVELOPMENTAL GENES IN *STREPTOMYCES*

***A. Chastanet**, D. B. Kearns, R. Losick;*
*Harvard University, Cambridge, MA*

The filamentous soil bacterium *Streptomyces coelicolor* undergoes a complex cyclic of morphological differentiation involving the erection of aerial hyphae that metamorphose into chains of spores. The regulatory cascade controlling this differentiation process involves numerous genes classified in two groups: the *bld* genes, which are required for the erection of aerial hyphae, and the *whi* genes, which are needed for converting aerial hyphae into spores. One useful approach to identifying additional genes involved in morphological differentiation has been insertional mutagenesis, which was carried out on a genome-wide scale (Gehring et al 2000). Although several developmental genes were identified in this manner, a complication is using this approach is the high background of spontaneous *bld* mutations in *S. coelicolor*. To circumvent this problem, we decided to use another strain, *Streptomyces venezuelae*, as a tool in which we generated *bld* mutants and selected candidates genes for interruption in *S. coelicolor*.

Indeed, *S. venezuelae*, which has been studied for years because of its ability to produce chloramphenicol (Cm) and jadomycin B (JdB), is an ideal candidate for this approach because it exhibits a robust developmental cycle and the frequency of spontaneous developmental mutations is low (J. Westpheling, personal communication). Based on the method described for *S. coelicolor*, we generated an insertionally mutated library of cloned chromosomal DNAs, introduced them in *S. venezuelae*, and recovered the transposon insertions. The characterization of the *S.venezuelae* mutants is still in progress, but we found two transposons in genes orthologous to *bldN* and *bldM* of *S. coelicolor* and other insertions in previously uncharacterized genes.

# A37

## SYSTEMATIC ANALYSIS OF TWO-COMPONENT SIGNAL TRANSDUCTION SYSTEMS REGULATING CELL CYCLE PROGRESSION AND DEVELOPMENT IN *CAULOBACTER CRESCENTUS*

***J. Skerker****, M. Prasol, B. Perchuk, E. Biondi, M. Laub;*
*Harvard University, Cambridge, MA*

Progression through the cell cycle requires the precise coordination of DNA replication, chromosome segregation, cell division, and cell growth. How these fundamental processes are coordinated and regulated can be studied in the model bacterial system *Caulobacter crescentus*. During each cell cycle, *Caulobacter* divides asymmetrically, to generate two distinct progeny; thus, cell cycle progression must also be coordinated with cellular differentiation. Previous genetic screens have identified 12 two-component signal transduction genes that control cell cycle progression or differentiation in *Caulobacter*, demonstrating the importance of this widespread class of regulatory molecules. However, the genome encodes 107 two-component signaling genes (63 histidine kinases and 44 response regulators) and the function of most remains unknown. To identify all two-component pathways controlling cell cycle progression or morphogenesis, we systematically deleted all 107 genes and performed a series of phenotypic tests to characterize each deletion strain. This global approach identified 36 mutant strains with cell cycle or developmental phenotypes, including all 12 previously characterized two-component genes. Four previously uncharacterized two-component genes were identified as essential for growth or viability of *Caulobacter*.

To complement our genetic studies, we developed a systematic biochemical technique, termed kinase-substrate profiling, which allows the rapid and accurate identification of the phosphorylation targets of histidine kinases. Using kinase-substrate profiling we identified a new essential two component pathway, between the orphan histidine kinase CenK and the orphan response regulator CenR. We demonstrate the existence of this pathway *in vivo* by analyzing depletion, over-expression, and co-expression strains. Depletion of either gene product disrupts membrane integrity, motility, and stalk formation. Over-expression of CenR leads to rapid cell lysis and death. We conclude that this new pathway (CenK - CenR) plays a role in peptidoglycan or cell membrane metabolism, and is the first example in Gram-negative bacteria of a two-component signaling system controlling these essential processes. A similar combination of genetics and kinase-substrate profiling is being used to systematically map other signal transduction pathways in *Caulobacter crescentus* affecting development and cell cycle progression.

# A38

## ROLE OF COILED-COIL DOMAINS IN THE OLIGOMERIZATION OF DIVIVA

*M. D. Rigden, S. Ramirez-Arcos,* ***N. F. Eng****, J. R. Dillon;*
*University of Ottawa, Ottawa, ON, CANADA.*

The involvement of DivIVA in cell division and chromosome segregation has been described in a number of Gram positive organisms. To date, studies of DivIVA have primarily focused on localization, overexpression or disruption of DivIVA in various bacterial backgrounds. We were interested in defining potential domains involved in the oligomerization of this protein and undertook an analysis of the coiled-coil domains of DivIVA from the Gram positive coccus *Enterococcus faecalis* . Bioinformatic analysis of 24 known DivIVA homologs, and *E. faecalis* DivIVA in particular, identified four distinct coiled-coil domains. With the exception of the C-terminal domain, the location and relative size of the domains were conserved across species. The involvement of each domain in $DivIVA_{Ef}$ oligomerization was determined using a combination of site directed mutagenesis and deletional analysis. Specifically, site directed mutations were used to disrupt the highly conserved N-terminal coiled-coil domain while deletional analysis was used to examine both the two central coiled-coil domains and the C-terminal coiled-coil domain. Disruption of either the N-terminal or deletion of the C-terminal coiled-coil domains resulted in no change in complex formation as determined by native gel electrophoresis and size exclusion chromatography, while deletion of the central coiled-coil domains of $DivIVA_{Ef}$ resulted in decreased complex formation. After identifying the two central coiled-coil domains as being involved in oligomer formation, further site directed mutational analysis was used to more precisely define the domains involved in oligomer formation and yielded a monomeric DivIVA. Yeast two hybrid studies of $DivIVA_{Ef}$ mutants demonstrating decreased and abrogated complex formation indicated that the two central coiled-coil domains interact preferentially with the opposite domain on another monomer. Based on the results of this study and the recently published transmission electron microscope image of the *Bacillus subtilis* DivIVA oligomeric complex, a Zipper model for DivIVA oligomerization is proposed.

# A39

## INVESTIGATING THE ROLE OF CTPA AND LON PROTEASES IN BACTEROID DEVELOPMENT IN *RHIZOBIUM LEGUMINOSARUM*

***K. Gilbert**, C. Tsui, C. K. Yost;*
*Biology Department, University of Regina, Regina, SK, CANADA*

Symbiotic nitrogen fixing members of the *Rhizobiaceae* can exist in two distinct cellular states: as free-living soil dwelling Gram negative rods, or as intracellular plant symbionts termed bacteroids. Bacteroid cells are significantly different in their morphology and physiology when compared to their free-living counterparts. The mechanisms controlling the bacteroid differentiation process are largely unknown. Proteases have been implicated in cellular differentiation and in host-bacteria interactions, however their role in bacteroid development is relatively uncharacterized. We have used a mutagenesis approach to investigate the roles Lon and CtpA proteases play in *Rhizobium leguminosarum* bacteroid development. Lon protease plays an important role in many host-bacteria interactions and in cellular development processes. A *lon* mutant of *R. leguminosarum* biovar *viciae* 3841 has been created using an insertional gene disruption technique. We have observed that the *lon* mutant still forms functional nodules and therefore Lon is not required for bacteroid development in *R. leguminosarum*. This is contrary to the nodulation minus phenotype observed in a *Sinorhizobium meliloti lon* mutant. Little is known about protein processing during bacteroid development, therefore we selected an uncharacterized C-terminal signal processing protease (CtpA) for further study. Repeated attempts to mutate *ctpA* have been unsuccessful, suggesting that it may be an essential gene in free-living conditions. Expression of *ctpA* was studied using *gusA* reporter gene constructs and the data suggests *ctpA* is strongly expressed in free-living conditions. Creation of a *gusA* fusion that is stably maintained in the nodule is underway to measure *ctpA* expression levels of *R. leguminosarum* when in the bacteroid state. Proteases are required for the regulation of many bacterial processes; we hope to further elucidate their role in bacteroid development.

# A40

## PHYSIOLOGICAL SUBSTRATES OF TMRNA

***J. H. Russell**;*
*Pennsylvania State University, University Park, PA*

tmRNA, encoded by the *ssrA* gene, is a small RNA that regulates translation in all bacteria by mediating the addition of a peptide tag to proteins before they are released from the ribosome. In *Caulobacter crescentus* and other species, tmRNA is required for development, but the substrates that are responsible for these phenotypes are not known. We have purified and identified over 150 tmRNA substrates from *C. crescentus* in an effort to understand the physiological role of tmRNA in bacterial development and to determine substrate specificity rules for tmRNA. A variant of SsrA was produced in which the final six codons of the peptide tag sequence were changed to encode histidine. These mutations eliminate the proteolytic epitopes normally present in the tag, and allow substrates to be purified using metal chelate chromatography. Substrates purified from cells producing SsrA-H6 were separated by SDS-PAGE and analyzed by mass spectroscopy. Nine substrates are enzymes involved in DNA replication, recombination, and repair, consistent with the known role for tmRNA in initiation of DNA replication. Twenty-four substrates are involved in protein synthesis or degradation, indicating that tmRNA affects total protein production and stability in the cell. Seventeen substrates are membrane proteins involved in transport, including eight TonB-dependent receptors, suggesting a role for tmRNA in regulating membrane protein production or membrane transport. Eighteen substrates are involved in energy metabolism, indicating that tmRNA may play a role in tuning the metabolic potential of the cell. These postulated roles for tmRNA will be confirmed by examining the effect of tmRNA on individual substrates and processes. To identify substrate selectivity rules for tmRNA, characteristics of the protein sequence, mRNA structure, and DNA sequence adjacent to the tagging site were examined for conserved motifs. No common features were found in the protein sequence, but a 21 base pair DNA motif was identified downstream of the substrate tagging sites. This motif may cause premature termination of transcription, resulting in an mRNA with no stop codon and thereby targeting the protein for tagging by tmRNA.

# A41

## EVIDENCE THAT FRUA IS A TRANSCRIPTIONAL ACTIVATOR OF *DEV*, AN OPERON REQUIRED FOR *MYXOCOCCUS XANTHUS* DEVELOPMENT

***P. Viswanathan**[1], A. Garza[2], D. Kaiser[3], L. Kroos[1];*
*[1]Michigan State University, East Lansing, MI, [2]Syracuse University, Syracuse, NY, [3]Stanford University, Stanford, CA*

*Myxococcus xanthus* undergoes multicellular development under starvation conditions. Development includes coordinated cell movements (rippling, streaming, and aggregation) and sporulation. All these behaviors are controlled by intercellular C-signaling via signal transduction pathways that include the FruA response regulator protein. FruA is also required for induction of developmental genes in response to C-signal, but direct targets of FruA have not been reported previously. Here, we show that the *dev* operon, whose expression is required for sporulation, appears to have two promoters, with $P_2$ initiating at -145 bp relative to $P_1$ initiating at +1, based on primer extension analysis of developmental RNA and on deletion analysis with transcriptional *lacZ* fusions. The deletion analysis also revealed an essential *cis*-acting element between -345 and -293 bp. This element is bound

by purified FruA-DBD-$His_6$ or FruA-$His_6$ (i.e., hexahistidine-tagged versions of the predicted DNA-binding domain or the full-length protein, respectively) in electrophoretic mobility shift assays (EMSAs). Binding was further localized to -332 to -306 bp. Four multiple-base-pair mutations (F1, F2, F3, and F4) were made in this region and the effects on *dev-lacZ* expression *in vivo* and FruA binding *in vitro* were compared. The F2 and F3 mutations eliminated expression and binding, while the F1 and F4 mutations reduced expression and binding. These results suggest that FruA (possibly phosphorylated FruA) binds upstream of the *dev* promoters and activates transcription from both promoters.

# A42

## FATE OF TMRNA-TAGGED PROTEINS: A PATHWAY TO NEW ANTIBIOTICS?

***B. J. Venters****, T. A. Naumann, A. R. Horswill, S. J. Benkovic, K. C. Keiler;*
*PSU, University Park, PA*

SsrA, or tmRNA, is a small RNA that adds a short peptide tag to the C terminus of nascent polypeptides on stalled ribosomes. This tagging pathway is required for development and virulence in many species, and in Caulobacter crescentus it is required for normal cell cycle progression. In both C. crescentus and E. coli, most SsrA tagged proteins are rapidly degraded. Their fate depends on interactions with at least 4 proteases and 2 proteolytic adaptor proteins. In E. coli, most tagged proteins targeted for degradation bind to the proteolytic adaptor protein SspB, which delivers the substrate to the ClpXP protease. ClpXP is essential in C. crescentus, and we have identified a protein, SmpD, that performs the same functions as SspB. SmpD binds to the SsrA-encoded peptide tag in vitro, and in a strain deleted for smpD the half-life of tagged proteins is increased 3.5-fold in vivo. mRNA profiling and Western blot experiments demonstrate that SmpD is cell-cycle regulated at the mRNA and protein level, with peak expression during the swarmer-to-stalked cell transition, coincident with the peak in SsrA levels. These results are consistent with a role for SmpD in facilitating the degradation of SsrA-tagged proteins. However, a strain deleted for smpD does not have the ssrA phenotype. Therefore, either degradation of tagged proteins is not important for the ssrA phenotype, or there are pathways redundant to SmpD. To test for the importance of degradation of SsrA-tagged proteins, we performed a genetic screen for small-molecule inhibitors of the proteolytic pathway. A library of cyclic peptides was produced in E. coli using SICLOPPS technology (split intein-mediated circular ligation of peptides and proteins), and those that inhibited the degradation of a GFP-SsrA reporter were isolated by FACS. Assays in vitro demonstrated that these cyclic peptides are bona fide inhibitors of SspB-ClpXP-mediated degradation of tagged proteins (KI = 15-18 uM). These inhibitors are specific for the degradation of tagged proteins because no inhibition was observed in the absence of SspB. Linear versions of the cyclic peptides, and cyclic peptides of unrelated sequence, did not inhibit SspB-ClpXP. Addition of the cyclic peptide inhibitors to cultures of C. crescentus or Bacillus subtilis killed the cells with minimum bactericidal concentrations of 250 and 500 uM respectively. The basis for this lethality is under investigation. These results suggest that cyclic peptide inhibitors of SsrA-tagged protein degradation may be broad specificity antibiotics.

# A43

## INTERSPECIES INTERACTIONS: EFFECTS OF BACILLUS SUBTILIS SURFACTIN ON STREPTOMYCES COELICOLOR AERIAL HYPHAE DEVELOPMENT

***P. D. Straight***[1]*, J. Willey*[2]*, R. Kolter*[1]*;*
*[1]Harvard Medical School, Boston, MA, [2]Hofstra University, Hempstead, NY*

Many microbes produce surfactants that confer multiple advantages on the producer organisms. Two examples are: (i) surfactin from B. subtilis, which allows the cells to raise aerial structures (1) and swarm on a solid surface (2); and (ii) SapB from S. coelicolor, which is required for aerial growth of hyphae (3). The mechanism of action for these compounds is linked to the ability of surfactants to reduce surface tension at air-water interfaces. Hence, the model for SapB function in Streptomyces aerial growth posits that SapB is required to reduce local surface tension in order for filaments to grow away from the substrate. Based on this model, we would predict that any compound with surfactant properties should foster aerial growth of hyphae. However, when we culture S. coelicolor in close proximity to B. subtilis, we observe an inhibition of Streptomyces' ability to raise aerial hyphae. We have demonstrated that surfactin is responsible for this phenotype by analyzing mutant strains of B. subtilis defective in surfactin production. Also, purified surfactin is sufficient to inhibit aerial growth of Streptomyces. We have tested this effect on several Streptomyces strains and found that surfactin affects aerial growth in multiple Streptomyces strains, and that multiple strains of B. subtilis can inhibit aerial growth. These observations suggest that surfactin may have specific effects on the production and function of surfactants produced for development of Streptomyces.

(1) Branda SS, et al., 2001. Proc Natl Acad Sci USA 98(20):11621-6.
(2) Kearns DB and Losick R, 2003. Mol Microbiol 49(3):581-90.
(3) Willey J, et al., 1991. Cell 65(4):641-50.

# A44

## CELL-CELL SIGNALING THROUGH REGULATED PROTEOLYSIS

***N. J. Campo****, D. Z. Rudner;*
*Harvard Medical School, Boston, MA*

Sporulation in *B. subtilis* involves the activation of a cascade of

alternative sigma factors in a stage- and compartment-specific fashion. Activation of these transcription factors is tightly regulated to ensure that events occurring in the mother cell are coordinated with events occurring in the forespore. The final alternative sigma factor in this cascade is sigmaK ($\sigma^K$), which directs late gene expression in the mother cell, including genes involved in the synthesis and assembly of the spore coat. A signal transduction pathway emanating from the forespore triggers activation of $\sigma^K$ by proteolysis of an inactive precursor protein, pro-$\sigma^K$. The processing of pro-$\sigma^K$ requires the mother cell membrane protein, SpoIVFB, which is a putative membrane-embedded $Zn^{++}$ metalloprotease. SpoIVFB is negatively regulated by two other integral membrane proteins, SpoIVFA and BofA. All three proteins reside in a multimeric membrane complex that localizes to the mother-cell membrane that surrounds the forespore. The processing of pro-$\sigma^K$ is triggered by a signal protein, SpoIVB (referred to as IVB), which is produced in the forespore and is secreted into the space between the mother-cell and forespore membranes. Little is known about how the IVB signaling molecule relieves the inhibition imposed on SpoIVFB by SpoIVFA and BofA. An attractive model is that IVB, which has recently been shown to have protease activity, cleaves one of the negative regulators. Cleavage of SpoIVFA or BofA would result in the activation of SpoIVFB and pro-$\sigma^K$ processing. We have reconstituted IVB protease activity in vitro and investigated potential substrates. We have found that SpoIVFA is efficiently cleaved by IVB. The cleavage site was identified by N-terminal peptide sequencing and uncleavable mutants were generated . Our results indicate that IVB-dependent cleavage of SpoIVFA is important for signaling in vivo. Using our in vitro assay, we are currently investigating what happens to the signaling complex after IVB-dependent cleavage of SpoIVFA.

# A45

## REGULATION OF A BACILLUS SUBTILIS MOBILE GENETIC ELEMENT BY INTERCELLULAR PEPTIDE SIGNALING

***J. M. Auchtung****, C. A. Lee, R. E. Monson, A. P. Lehman, A. D. Grossman;*
*Massachusetts Institute of Technology, Cambridge, MA*

Horizontal gene transfer significantly contributes to the evolution of bacterial species. Mobile genetic elements play an important role in horizontal gene transfer, and characterization of the regulation of these elements should provide insight into conditions that promote horizontal gene transfer. We have characterized a newly identified mobile genetic element, ICE*Bsu1*, in the gram-positive bacterium *Bacillus subtilis*. ICE*Bsu1* is a member of the family of mobile genetic elements known as integrative and conjugative elements (ICEs), which are also known as conjugative transposons (CTns). We observed that ICE*Bsu1* transfers to *Bacillus* and *Listeria* recipient cells. We also found that transfer of ICE*Bsu1* is regulated by a self-encoded peptide signal and the global DNA damage (SOS) response. The self-encoded peptide signal acts to limit transfer into recipient cells that already contain ICE*Bsu1*. The global DNA damage response activates ICE*Bsu1* transfer; this may provide a mechanism for the element to escape the distressed host cell for a more suitable host. The peptide signaling molecule encoded by ICE*Bsu1* is a member of a family of signaling peptides found in *Bacillus* species and on other *Bacillus* mobile elements. Therefore, we hypothesize that peptide signaling may also regulate the activity of additional *Bacillus* mobile genetic elements.

# A46

## IDENTIFYING NOVEL COMPONENTS AND REGULATORS OF THE *ESCHERICHIA COLI* DIVISION APPARATUS USING A SYNTHETIC LETHAL SCREEN

***T. G. Bernhardt****, P. de Boer;*
*Case Western Reserve University, Cleveland, OH*

Cell division in bacteria is carried out by a multi-component molecular machine referred to as the septal ring. Assembly of the septal ring in *Escherichia coli* (and many other bacteria) starts with the polymerization of the FtsZ protein into a ring structure just beneath the cell membrane. Proper positioning of the division site is achieved by two partially redundant negative regulators of FtsZ ring assembly, the Min system and nucleoid occlusion (NO). The Min system is relatively well understood and is thought to restrict FtsZ polymerization to midcell by promoting the rapid pole-to-pole oscillation of an antagonist of FtsZ polymerization (MinC). In contrast to Min, the molecular mechanism(s) preventing FtsZ ring formation in the vicinity of the nucleoid resulting in the observed NO phenomenon have long remained mysterious.
Despite suffering a significant number of nonproductive polar divisions, cells lacking the Min system have a nearly normal growth rate. However, Margolin and co-workers have found that cell growth is severely affected when *min* mutations are combined with the thermosensitive *fts*Z84 alelle, or with a *rodA* mutation resulting in the loss of rod-shape. In addition, we suspected that the Min system might also become essential, or nearly so, in cells defective for NO. Thus, we developed a screen for mutations synthetically lethal with a Min defect (*slm* mutants), reasoning that it would be useful for the identification of (novel) factors involved in cell division, cell shape, and possibly NO. Using this screen, mutants affecting all three processes were isolated, and a novel septal ring component (EnvC) and the first NO factor in *E. coli* (SlmA) were identified. Details of the screen and the properties of the mutants will be discussed.

# A47

## CELL CURVATURE IN *CAULOBACTER CRESCENTUS*: A COLLABORATIVE EFFORT BETWEEN MREB AND CRESCENTIN

***G. Charbon**, C. Jacobs-Wagner;*
*Yale, New haven, CT*

Actin, tubulin and intermediate filament (IF) proteins constitute the main cytoskeleton elements determining the shape of eukaryotic cell. Recent data strongly suggest that equivalents of those cytoskeletal proteins exist in bacteria with MreB, FtsZ and crescentin being representative of the counterparts of actin, tubulin, and IF protein respectively. In addition, it has been shown that MreB and crescentin form in vivo filamentous structures critical to determine the shape of the bacterium *Caulobacter crescentus*. To unravel the mechanism by which crescentin determines the crescent and helical shapes of *C. crescentus*, we identified putative interacting partners of crescentin among, which was MreB. Furthermore, we provided genetic and cell biological evidence suggesting that MreB was involved in cell curvature by interacting, directly or indirectly, with crescentin. These findings are particularly interesting because they are reminiscent of the known interaction between actin and intermediate filaments in eukaryotes.

# A48

## LINKING GENE TRANSCRIPTION TO MORPHOGENESIS.

*T. Doan, K. Marquis, **D. Z. Rudner**;*
*Harvard Medical School, Boston, MA*

Through out the process of sporulation the forespore and mother cell follow completely different programs of gene expression but these programs are linked through signal transduction pathways. $\sigma^K$ directs late gene expression in the mother-cell compartment and its activity is tightly regulated to ensure that transcription of these genes is held in register with events occurring in the forespore. $\sigma^K$ is synthesized as an inactive precursor protein (pro-$\sigma^K$) and is activated by proteolytic processing. The conversion of pro-$\sigma^K$ to $\sigma^K$ requires the mother cell membrane protein SpoIVFB, which is likely to be the processing enzyme. SpoIVFB is held inactive by two other integral membrane proteins, SpoIVFA and BofA. All three proteins reside in a multimeric membrane complex that localizes with exquisite specificity to the mother-cell membrane that surrounds the forespore (the outer forespore membrane). The processing of pro-$\sigma^K$ is triggered by a signal protein, SpoIVB, which is produced in the forespore under the control of $\sigma^G$ and secreted into the space between the mother-cell and forespore membranes where it overcomes SpoIVFA and BofA inhibition of SpoIVFB. We have recently identified four mother cell proteins (SpoIID, SpoIIP, SpoIIM, SpoIIIAH) and one forespore protein (SpoIIQ) that are all required to efficiently anchor the pro-$\sigma^K$ signaling complex in the outer forespore membrane. We have investigated whether these five proteins play a *direct* role in the pro-$\sigma^K$ processing pathway or only participate indirectly by anchoring the signaling complex. All five anchoring proteins are required at earlier stages in the sporulation pathway (IID, IIP, IIM, and IIQ are required for engulfment and all five proteins are required for $\sigma^G$ activation). Thus, in order to investigate the role of these proteins in the $\sigma^K$ signaling pathway, we used a collection of $\sigma^G$ bypass mutants. Suprisingly, we have discovered that all five proteins are required for efficient pro-$\sigma^K$ processing. Interestingly, these proteins appear to act at different steps in the signaling pathway. These results suggest that $\sigma^K$ activation is coupled to both the activation of $\sigma^G$ and the morphological process of engulfment.

# A49

## TMRNA IS REQUIRED FOR PLASMID MAINTENANCE IN *CAULOBACTER CRESCENTUS*

***D. M. Lee**, S. Hong, P. Tee, K. C. Keiler;*
*Pennsylvania State University, University Park, PA*

The *ssrA* gene encodes tmRNA, a transfer-messenger RNA that acts on ribosomes stalled during translation, tagging the nascent polypeptide for proteolysis. Deletion of *ssrA* in *Caulobacter crescentus* results in a delay of DNA replication initiation. Additionally, deletion of *ssrA* or *smpB,* which encodes a protein that protects tmRNA from degradation, causes a disruption of plasmid maintenance. This plasmid maintenance phenotype is of interest since tmRNA is present in all sequenced bacterial genomes, and plasmids are known to harbor genes important in development and virulence. Several broad host range plasmids including members of the pBBR and RSF1010 families are able to replicate in wild type *C. crescentus*. However, strains lacking tmRNA activity due to loss of *ssrA* or *smpB* can no longer be stably transformed with any of these plasmids, unless the plasmid carries a gene that complements the chromosomal mutation. Plasmids containing the *C. crescentus* origin of replication are maintained in cells lacking tmRNA activity, indicating that there are no gross defects in plasmid physiology. Two selection strategies were used to find suppressors of the plasmid maintenance phenotype. A pBBR1-based plasmid was mutagenized, and mutations that bypass the need for tmRNA activity were selected. A deletion that results in a truncation of the C-terminal end of Rep, the origin-binding protein of pBBR1, allows the plasmid to be maintained in the presence or absence of tmRNA activity. Wild-type Rep was also identified as a substrate for tmRNA. These data led to the hypothesis that Rep must be processed by tmRNA for plasmid replication to proceed normally. tmRNA may also interact with proteins encoded by chromosomal genes to control plasmid

maintenance. A selection for chromosome-based mutations that allow maintenance of pBBR1-based plasmids has revealed a suppressor of the plasmid maintenance phenotype. This second-site suppressor grows at the same rate as the *ssrA* deletion strain, indicating that it does not suppress the chromosome replication phenotype. These data imply that the DNA replication initiation delay and the plasmid maintenance phenotypes are caused by distinct regulatory effects rather than a global defect, and suggest that tmRNA may have specific roles in DNA replication initiation and plasmid maintenance that are separate from protein quality control.

# A50

## EVIDENCE FOR A SHORT-RANGE SIGNALING SYSTEM INVOLVED IN ACTIVE SEEDING DISPERSAL FROM *PSEUDOMONAS AERUGINOSA* BIOFILMS

*L. Purevdorj-Gage[1], G. Ehrlich[2], J. Costerton[3],* ***P. Stoodley[2]****; [1]Montana State University, Bozeman, MT, [2]Center for Genomic Sciences, Pittsburgh, PA, [3]University of Southern California, LA, CA*

Biofilm formation is a common feature of prokaryotes and is particularly well documented within the proteobacteria. Microscopic and molecular techniques have revealed that biofilm formation is a complex multifactorial process regulated by both genetic and environmental factors. Morphological evidence also suggests that biofilms may form rudimentary fruiting bodies for active "seeding" from a surface. We used a flow cell system to investigate the formation and differentiation of seeding by *P. aeruginosa* biofilms in wild type (WT) non mucoid (PAO1) and mucoid (FRD1) strains, and in various PAO1 strains with mutations in known genes associated with biofilm development. After 3 days growth of the PAO1 WT biofilms we observed that cells within the centers of the larger microcolonies became highly motile, while the cells on the outside formed a non-motile "wall". The motile cells then evacuated the microcolony through gaps in the wall, leaving behind empty shells which gave a characteristic "donut" appearance with bright field microscopy. We termed this phenomenon "seeding dispersal" and found that a threshold microcolony diameter of approximately 80 μm was required for seeding to occur, suggesting a role for quorum sensing (QS) in the process. Seeding did not occur in biofilms grown from FRD1 or the las/rhl QS mutant JP2, but did occur in PA01- Δ*rhlA*, a rhamnolipid mutant. We developed a technique to induce the high motility associated with seeding by growing colonies on roughened agar poured onto a glass slide. The phenotype did not occur on smooth agar surfaces. In the roughened agar assay we could induce motility without relying on the precursory step of full microcolony formation by stimulating high cell density. We found that WT, JP1, JP2, and PA01-Δ*rhlA* were positive for seeding motility but FRD1 was negative. We found that a threshold packing density of 0.05 cells / $\mu m^2$ was required to initiate seeding behavior. Our results suggest that a yet unidentified signaling system along with the las/rhl system may coordinate microcolony development and subsequent seeding. We have identified a candidate gene associated with seeding dispersal and preliminary results using a knockout mutant appear to abolish the emptying of the colony. Identification of pathways which control dispersal in the proteobacteria, which contain many biofilm forming pathogens, is of both academic and practical interest.

# A51

## SUBSTRATE REQUIREMENTS FOR REGULATED INTRAMEMBRANE PROTEOLYSIS OF PRO-SIGMA$^K$

***R. Zhou****, L. Kroos;*
*Michigan State University, East Lansing, MI*

Regulated intramembrane proteolysis (RIP) is an important mechanism for controlling signaling pathways in both prokaryotes and eukaryotes. Proteases that carry out RIP are called intramembrane-cleaving proteases (I-CLiPs). These proteases catalyze hydrolysis of their substrates within a membrane or near its surface. How water gains access to the active site of I-CLiPs is unclear. Likewise, it is unclear how I-CLiPs access their substrates, which are typically α-helical around the cleavage site, a conformation expected to prevent nucleophilic attack on peptide bonds due to steric hindrance by amino acid side chains. Also, little is known about how I-CLiPs recognize their substrates. To address these questions, we are studying RIP of pro-$\sigma^K$ by *Bacillus subtilis* SpoIVFB, an I-CLiP in the S2P family. In response to a signal from the forspore, SpoIVFB cleaves pro-$\sigma^K$ after amino acid 20, releasing active $\sigma^K$ from the outer forspore membrane into the mother cell cytosol. We showed previously that nearly half of pro-$\sigma^K$ (amino acids 1 to 117) is sufficient for RIP, but amino acids 1 to 109 was insufficient. Also, we showed that RIP is accurate and abundant when SpoIVFB and pro-$\sigma^K$(1-126)-H6 (i.e., a C-terminally truncated substrate tagged with hexahistidine) are overproduced in *E. coli*. Using this *E. coli* system, we now report that a triple alanine substitution at positions 114-116 of pro-$\sigma^K$(1-126)-H6 nearly eliminated RIP, whereas substitutions nearby (i.e., single amino acid substitutions at 109-113 or 117) had no effect. Also, loss of charge at position 13 in the pro-sequence (K13A) or at position 23 (K23A) near the cleavage site nearly eliminated RIP, and a double substitution (N24D and P28A) or a single change (P28A) eliminated RIP. These results suggest that amino acids 114-116 of pro-$\sigma^K$ (or the α-helix in which they are predicted to lie) are a key determinant in substrate recognition. This might be mediated by the C-terminal cytosolic part of SpoIVFB since deletion of 9 amino acids from the C-terminus abolished RIP. Positive residues at positions 13 and 23 of pro-$\sigma^K$ are critical. Arginine can substitute for lysine at these positions. We speculate that these positive residues might help bring water to the active site of

SpoIVFB within the membrane or at its interface with the mother cell cytosol. Further, helix-breaking residues at positions 24 and 28 of pro-$\sigma^K$ might help prevent α-helix formation, allowing SpoIVFB access to the cleavage site.

# A52

## EFFECTS OF PENICILLIN BINDING PROTEINS ON OSCILLATION OF MIN PROTEINS AND CELL SEPARATION IN ABERRANTLY SHAPED AND BRANCHED CELLS OF ESCHERICHIA COLI

***K. D. Young****, A. Varma, R. Priyadarshini;*
*Univ. of North Dakota School of Medicine, Grand Forks, ND*

We are studying the mechanisms underlying the generation of normal cell shape in E. coli, with a special emphasis on the contributions of the low molecular weight penicillin binding proteins (LMW PBPs). Recently, we established that FtsZ and PBP 5 collaborate to generate a uniform cell shape. Surprisingly, by manipulating this interaction E. coli can be induced to grow as a spiral shaped filament. Therefore, we examined if FtsZ polymerized abnormally in aberrantly shaped or branched cells. The localization of FtsZ-GFP was virtually normal in cells having minor shape defects (bends, kinks, non-uniform diameter). However, in a strain lacking 7 LMW PBPs, aberrant FtsZ rings were found in 8-10% of cells exhibiting a severely compromised morphology or in cells with two or more branches. Because proper Z ring placement depends on the MinCDE proteins, we observed the oscillation of these proteins in aberrantly shaped and branched cells. In branched cells having three or more visibly distinct poles, GFP-MinC oscillated in repetitive cycle, most often by moving clockwise or anti-clockwise from pole to pole in turn. Surprisingly, the behavior of MinE-YFP did not parallel this motion by MinC. Instead, MinE-YFP oscillated from one half of a branched cell to the other half. It appears that in branched and misshapen cells the movement of MinE and MinC are not coordinated in a simple manner.
In separate experiments, we determined whether PBPs 4 and 7, endopeptidases that hydrolyze the peptide crosslinks between peptidoglycan chains, might play a role in septum cleavage and cell separation. As more LMW PBPs were deleted from strains carrying mutations in the peptidoglycan amidases (AmiA, AmiB or AmiC), the percentage of cells in chains increased in ΔamiC mutants, indicating that one or more PBPs help separate newly divided cells. The longest chains were observed in cells lacking multiple LMW PBPs, but the major effects were mediated by deleting PBPs 4 and 7. Although the loss of PBPs 4 and 7 had no effect on cell separation when these PBPs were deleted individually or together, when combined with an amiC mutation the deletion of either PBP 4 or 7 increased chain lengths 10-20% beyond that caused by the loss of amiC alone. These results represent the first identified physiological functions for PBPs 4 and 7, and suggest that these LMW PBPs play accessory roles in cleaving peptidoglycan during cell septation.

# A53

## THE ROLE OF PODJ PROCESSING IN SWARMER PROGENY-SPECIFIC DEVELOPMENT

***E. M. Quardokus****, Y. V. Brun;*
*Indiana University, Bloomington, IN*

Caulobacter crescentus is an aquatic, dimorphic, Gram negative, alpha-purple proteobacterium that undergoes a developmental lifecycle. During a normal cell division cycle, swarmer cells differentiate into stalked cells and stalked cells reproduce to yield a new, motile progeny swarmer cell. Polar development occurs in an ordered series of steps: flagellum assembly and flagellum activation occur before cell division whereas, pili assembly, flagellum ejection, pili retraction, holdfast assembly and stalk synthesis occur after division, in the next swarmer cell cycle. This succession of polar developmental events is referred to as swarmer progeny-specific development (SwaPS). If cell division is blocked, the swarmer cell differentiates into a stalked cell, the stalked cell replicates its chromosome and becomes a stalked predivisional cell, but halts development of the new swarmer pole just after flagellum assembly; activation of flagellum motility, development of pili, holdfast and stalk synthesis are blocked. The paralyzed flagellum phenotype is similar to that of null mutants of the histidine kinase/phosphatase PleC. Providing constitutively phosphatase active alleles of PleC to cell division blocked cells restore motility and stalk development. PleC relies on the polar organelle development protein PodJ for targeting its localization to the new swarmer pole and its full activity. Here we focus on the relevance of PodJL processing and the domains of PodJ with respect to their roles in SwaPS and the cell division checkpoint for development. We identify regions of PodJ that are important for blocking flagellum activation when cell division is inhibited and for the localization of the response regulator DivK, which is known to block SwaPS in predivisional cells.

# A54

## UNVEILING THE MYSTERY OF LPP-CELL WALL LIGASE

*S. Weinert,* ***Y. N. Yu****;*
*Max-Planck Institute for Developmental Biology, Tuebingen, GERMANY*

Murein lipoprotein (Braun Lpp) is the most abundant outer membrane protein in the enterobacterium Escherichia coli, with ~750,000 molecules per cell. Since its discovery 36 years ago, much has been learned about the biosynthesis, lipid modification and translocation of Lpp. However, the genes

responsible for the most unique feature of Lpp - covalent crosslinkage to the cell wall - have remained enigmatic. By anchoring to the inner leaflet of the outer membrane with its N-terminal cysteine lipid moiety and binding covalently to the peptidoglycan at its C-terminal lysine residue, Lpp creates a nano-suspender-like structure that is evenly distributed throughout the cell envelope. Retention of Lpp to the inner membrane is tolerable but cross-linkage of this mislocated protein to the cell wall leads to cell lysis. Thus, Lpp functions to insure proper "hanging" of the cell wall to the outer membrane and is important to preserve bacterial envelope architecture and function. The Lpp-cell wall attachment is presumably generated by a periplasmic transpeptidase that catalyses a peptide bond between the ε-amino group of the Lpp C-terminal lysine and the L-center of meso-diaminopimelate (Dap) residue of a murein donor tetrapeptide stem. Four paralogous genes (ybiS, ycfS, erfK and ynhG) in E. coli contain a wall binding motif, LysM domain and the conserved YbiS/YcfS/ErfK/YnhG domain. In this report we show that a quadruple knockout of these paralogs results in cells with only the free, unlinked form of Lpp. Individual reintroduction of ybiS, ycfS or erfK into the quadruple mutant restores Lpp-cell wall transpeptidation. Our results not only identify the genes encoding the Lpp/cell wall ligase but also assign a transpepdidatrion function for the widespread eubacterial YbiS/YcfS/ErfK/YnhG homologs.

# A55

## OVEREXPRESSION OF *FTSA* AND *FTSZ* IS SUFFICIENT FOR THE STABLE MORPHOGENESIS OF *ESCHERICHIA COLI* FROM A ROD TO A SPHERE, WITH A KEY ROLE FOR FTSW

***M. M. Khattar**, D. A. Mansour, L. Abdul-Latif, Z. W. El-Hajj;*
*American University of Beirut, Beirut, LEBANON*

In the gram-negative bacterium *Escherichia coli*, Penicillin-Binding Protein 2 (PBP2; encoded by *phpA*) is required for the maintenance of the rod shape. Inhibition of PBP2 using the specific inhibitor mecillinam, or the inactivation of *phpA*, results in the loss of the rod shape in favour of spherical morphology. In either case, the rod-to-sphere morphogenesis precipitates a lethal failure in cell division which is readily overcome by overexpression of the essential cell division genes *ftsQ*, *ftsA* and *ftsZ* from the multicopy plasmid pZAQ. Here we show that overexpression of the cell division genes *ftsQ*AZ using a low-copy number plasmid (pBS58) supports the successful inactivation of *phpA* and the viability of resulting spherical *E. coli*, as observed previously using plasmid pZAQ. Furthermore, a derivative of pBS58 (pΔB) in which the N-terminus of the *ftsQ* gene is deleted, supported the inactivation of *phpA* in a manner that was sensitive to medium conditions and genetic background, and in particular the *relA* gene, suggesting a redundancy of *ftsQ* overexpression under some conditions. Interestingly, overexpression of *ftsW* restored the ability of a *relA* mutant of *E. coli* to accept the *phpA* deletion to the normal levels observed in the *relA*$^+$ parent strain, when only *ftsA* and *ftsZ* were overexpressed. Additionally, an *ftsW201*(Ts) mutant strain carrying plasmid pBS58 did not accept a *phpA* deletion under permissive conditions. Our findings suggest strongly that the putative division-specific transglycosylase, FtsW, can compensate for insufficient FtsQ in spherical cells under certain conditions and that it is likely to be a key component that is over-activated by FtsQAZ to ensure a permanent switch from lateral to septal murein synthesis during the rod-to-sphere morphogenesis in *E. coli.*

# A56

## REAL-TIME PCR ANALYSIS OF GENOME COPY NUMBER IN INDIVIDUAL *EPULOPISCIUM* CELLS

***J. E. Mendell**, E. R. Angert;*
*Cornell University, Ithaca, NY*

Currently, *Epulopiscium* spp. are the largest heterotrophic bacteria described, with some cigar-shaped cells reaching lengths in excess of 600 μm. These low G+C Gram-positive bacterial symbionts are found in the intestinal tracts of certain surgeonfish (Family Acanthuridae). In addition to its unusually large size, an *Epulopiscium* cell contains an enormous amount of DNA. The *Epulopiscium* genome has not been well characterized, primarily because these organisms cannot yet be grown in pure culture. We hypothesize that the DNA in an *Epulopiscium* cell is comprised of a small, approximately 4 Mb genome, which is present in thousands of copies per cell. To investigate genome copy number in *Epulopiscium* we have developed real-time TaqMan PCR assays which allow us to quantify specific genes in individual *Epulopiscium* cells. Several unlinked, presumptive single-copy genes, used to represent the unit genome of *Epulopiscium*, have been assayed. The results from the majority of these markers indicate that a large *Epulopiscium* cell contains tens of thousands of copies of its genome. This number of genome copies per individual bacterium is unprecedented and may represent a significant adaptation that allows an *Epulopiscium* cell to maintain its large cell volume.

# A57

## CONTROL OF CHROMOSOME SEGREGATION AND CELLULAR DIFFERENTIATION IN *CAULOBACTER CRESCENTUS*

***R. B. Jensen**;*
*Roskilde University, Roskilde, DENMARK*

In *Caulobacter crescentus*, progression through the cell cycle is

coupled to a cellular differentiation program. The replicationally quiescent motile swarmer cell possesses a single copy of the chromosome, which is oriented with the origin-proximal region located close to the flagellated pole of the cell. DNA replication initiates at the swarmer-to-stalked cell transition and one of the newly replicated origins move to the opposite pole of the cell shortly after replication is initiated. A very short delay between initiation of DNA replication and origin movement is observed, indicating either absence of or only a very brief period of cohesion between the newly replicated origin-proximal parts of the *Caulobacter* chromosome. The DNA replication apparatus forms a mobile factory during replication. Initially, the replication factory is located at the stalked pole of the cell and as replication proceeds, the replication apparatus gradually moves to midcell. The terminus region of the chromosome gradually moves from the pole opposite the stalk to midcell during the DNA replication process. The completely replicated terminus regions stay associated with each other for an extended period of time after chromosome replication is completed. The terminus regions disassociate shortly before the dividing cells separate. Invagination of the cytoplasmic membrane is observed before the terminus regions separate, resulting in trapping of a chromosome on either side of the cell division septum. Cytoplasmic membrane invagination takes place approximately 20 minutes before separation of the progeny cells, during the final step of the cell division process. The uncoupling between invagination of the cytoplasmic membrane and the outer part of the cell wall could be responsible for the previously observed compartmentalization of the predivisional cell. A defect in either completion of DNA replication or chromosome segregation results in inhibition of the final separation of the dividing cell, giving an arrested deeply pinched predivisional cell.
The combination of directed movement of the origin-proximal part of the chromosome by a force-generating filament, anchoring of the chromosome to the cell poles, movement of the bulk of the chromosomal DNA by the replication factory and chromosome condensation is likely to be responsible for chromosome segregation in *Caulobacter*.

# A58

## DIRECTIONAL CONTROL OF MOTILITY IN M. XANTHUS: REGULATED POLE-TO-POLE OSCILLATIONS OF AN ESSENTIAL MOTILITY PROTEIN

***T. Mignot**, J. Merlie, D. Zusman;*
*UC Berkeley, Berkeley, CA*

The maturation of organized bacterial communities and the development of higher eukaryots require that individual cells coordinate their motility to build differentiated multicellular structures. In both cases, this coordination relies on mechanisms involving cell-to-cell extracellular signaling and the establishment of cell polarity. The developmental scheme of the gram-negative bacterium Myxococcus xanthus requires that individual cells organize their direction of movement so as to move coordinately towards the aggregation centers that will mature as fruiting bodies. Cells periodically reverse their direction of movement so that the leading pole becomes the lagging pole. The frequency of cellular reversals is dependent on the Frz system, a chemotaxis-like signal transduction pathway that regulates directional motility by a process thought to involve coordinated polar switches of two distinct motors. However, evidence for this model is still lacking. Here, we provide evidence that the FrzS, a protein with a novel modular structure consisting of an N-terminal receiver-like domain fused to an extended coiled-coil domain, may be a determinant of directional motility. FrzS localized to the cell poles as organelle-sized hollow cylindrical structures. Site-directed mutagenesis identified a localization signal critical for function at the extreme C-terminal end of the protein. In moving cells, FrzS shuttled from pole-to-pole and oscillations were correlated with the direction of movement. We show that the frequency of oscillations is regulated by the signaling activity of the Frz pathway. Interestingly, movement analysis suggests that pole-to-pole migration of FrzS does not result from diffusion but rather involves tracking along a coiled filament that runs along the cell length. We discuss these results in the general context of controlled cell polarity and the bacterial cell architecture.

# A59

## SIGF, AN ECF SIGMA FACTOR MEDIATING THE RESPONSE TO HYDROGEN PEROXIDE DURING STATIONARY PHASE IN CAULOBACTER CRESCENTUS

*C. E. Alvarez-Martinez, R. L. Baldini, **S. L. Gomes**;*
*Instituto de Química, Universidade de São Paulo, São Paulo, BRAZIL*

Alternative sigma factors of the ECF subfamily (extracytoplasmic function) are important regulators of stress responses in bacteria, and have been implicated in the control of homeostasis of the extracytoplasmic compartment of the cell. This work describes the characterization of *sigF*, encoding one of the thirteen members of this subfamily identified in *Caulobacter crescentus*. A *sigF* null mutant was obtained and analyzed under various stress conditions, and shown to be severely impaired in resistance to hydrogen peroxide treatment, exclusively during the stationary phase of growth. SigF protein levels increase significantly during stationary phase due to a post-transcriptional control, as there is a decrease in the transcription rate of *sigF* gene during this growth phase. The stability of SigF protein was shown to be low during exponential growth but quite high during stationary phase, and FtsH protease seems to be directly or indirectly involved in the control of SigF half-life. Microarray experiments led to the identification of eight genes regulated by SigF during stationary phase, including *sodA* and *msrA*, which are known to be involved in oxidative stress response.

This is the first characterization of an ECF sigma factor in *C. crescentus*.

# A60

## IDENTIFICATION OF GENES ASSOCIATED WITH BACTERIOCIN PRODUCTION IN *STREPTOCOCCUS MUTANS* BY RANDOM INSERTIONAL MUTAGENESIS

***P. W. Tsang***, *J. Merritt, T. Nguyen, W. Shi, F. Qi;*
*UCLA, Los Angeles, CA*

The dental biofilm, also known as the dental plaque, is perhaps one of the most complex multispecies microbial communities. It consists of over 500 different microbial species. These different species normally coexist in a homeostatic state. However, under environmental perturbation, this balance may be disrupted, leading to diseases such as dental caries and periodontal diseases. *Streptococcus mutans* is one of the major pathogens implicated in dental caries. It is suspected that the production of bacteriocins (mutacins), which inhibits the growth of other gram-positive commensal bacteria, may play an important role in *S. mutans*' persistence in the dental biofilm. Previous studies from our laboratory have demonstrated that mutacin production is modulated by various environmental factors. It has also been shown that the level of mutacin production influences *S. mutans'* competition with other streptococcal species. Global regulators, such as *ciaH & luxS,* have also been implicated in the regulation of mutacin production. As an initial attempt to understand the intricate regulatory pathways of mutacin gene expression, we used a random insertional mutagensis approach to search for genes that are associated with mutacin I production in the virulent strain UA140. A random insertional mutagenesis library consisting of 11,000 clones was constructed and screened for mutacin defective mutants. Seventy mutacin defective clones were isolated and their mutational sites were determined by PCR amplification followed by sequencing. A total of 25 unique genes have been identified. These genes can be categorized into the following classes: two-component sensory systems, stress response, energy metabolism and central cellular processes. Several conserved hypothetical proteins with unknown functions were also identified. These results suggest that mutacin production is controlled stringently by diverse and complex regulatory pathways. The interconnection and hierarchy of these genes are currently under investigation.

# A61

## IRON ACQUISITION BY SINORHIZOBIUM MELILOTI IN SYMBIOSIS WITH THE HOST PLANT MEDICAGO SATIVA AND IN THE FREE LIVING STATE

**M. O'Connell**, P. O Cuiv, C. Viguier, D. Keogh, P. Clarke;
Dublin City University, Dublin, IRELAND.

A number of iron rich molecules, including nitrogenase and leghaemoglobin, function in the nitrogen fixing symbiosis between rhizobia and their host plants. Moreover, in the free living state rhizobia must compete with other soil microbes for the limited iron available. The mechanisms by which iron is acquired by *Sinorhizobium meliloti*, the endosymbiont of *Medicago sativa* (alfalfa), were investigated. The model strain of the species produces a siderophore, rhizobactin 1021. The genetic basis for the synthesis, transport and regulation of the siderophore has been determined. In contrast to what has been observed for iron response regulation of siderophore production and transport in other gram negative bacteria, the rhizobactin regulon is not regulated by Fur but repressed by a novel regulator RirA. The role of this regulator has only been confirmed in one other species, *Rhizobium leguminosarum*. The reason why rhizobia have replaced Fur with a novel regulator of the iron response is unclear. The AraC-like regulator RhrA also positively regulates the rhizobactin regulon. Furthermore, at least one of the biosynthesis genes is upregulated by luteolin present in the host plant exudate. Genes of the rhizobactin regulon are not expressed in the symbiotic state at the time when nitrogenase is being synthesised. The mechanism by which the endosymbiont satisfies its iron requirement during symbiosis remains to be understood.

# A62

## CRYO-ELECTRON-TOMOGRAPHY OF THE CHEMOTACTIC SYSTEM OF THERMOTOGA MARITIMA

***A. Briegel***[1]*, M. Gruska*[2]*, J. Plitzko*[2]*, H. Engelhardt*[2]*, W. Baumeister*[2]*;*
*[1]California Institute of Technology, Pasadena, CA,* [2]*MPI, Martinsried, GERMANY*

Chemotaxis, the ability of organisms to sense and to respond to their surrounding environment, is widespread among the prokaryotes. This two-component signalling pathway has been well studied and the structure of most of the components of this system are solved already. But despite this knowledge, the arrangement of these macromolecules in the intact cell is largely unknown. Until now, the imaging techniques were not sufficient to visualize the assembly of the chemoreceptor clusters or the flagellar motor in intact cells.
Cryo-electron tomography provides the unique opportunity to investigate intact prokaryotic cells in a near-to-native state at molecular resolution. Using automated data acquisition schemes, vitrified samples may be viewed in the electron microscope with minimal radiation damage . Here, we applied cryo-electron tomography to the bacterium Thermotoga maritima to get insights into its supramolecular architecture. Whole cells were embedded in vitreous ice by plunge freezing. These vitrified specimens are essentially free of artifacts, as neither dehydration nor staining are necessary for preservation

or for contrast enhancement. Single-axis tilt series were recorded under low dose and cryo conditions using the Philips CM300 FEG transmission electron microscope (TEM) operated at 300kV and additionally equipped with a Gatan imaging filter (GIF). Once a 3-D data set (tomogram) is recorded and processed it can be sectioned in silico in any direction desired to display and analyze structural details of the cell's interior. Rendering techniques allow us to visualize these structural features in 3-D.
The focus of our work was the structural components of the chemotactic system of Thermotoga. The interplay of attractants and repellents with the chemoreceptors, the methyl-accepting proteins (MCPs), CheA (a kinase), CheW (linking protein), and CheY control the rotation of the flagella. With cryo-electron tomography we studied the major components of this system in situ, including the sensory part of this system, the chemoreceptor clusters, as well as the executive part, the flagellar motor. In particular, we identified the cluster of chemoreceptors near the cell pole, discovered their arrangement and characterized the flagellar motor, which we found to have an uncommon basal body structure.

# A63

## CONSEQUENCES OF BLOCKED CTRA BINDING TO A CHROMOSOME REPLICATION ORIGIN

***P. Bastedo**, G. T. Marczynski;*
*McGill University, Montreal, PQ, CANADA*

Cell division in *Caulobacter crescentus* is asymmetric. An adherent, "stalked" cell is replication-competent, while the motile "swarmer" cell is blocked in chromosome replication until after differentiation into the "stalked" cell type. Binding of the response regulator CtrA to five sites (a-e) in the chromosme replication origin (*Cori*) has been implicated in this swarmer-specific repression of replication; CtrA is abundant and phosphorylated in swarmer cells, and proteolytic clearance of the protein during development into stalked cells coincides with the initiation of chromosome replication. Directed mutagenesis of the replication origin was performed to test this simple model of replication control. Plasmids that depend on cloned *Cori* DNA for autonomous replication suggest that CtrA binding to *Cori* does more than simply repress replication, since mutation of individual CtrA binding sites can either improve or impair autonomous replication. Such mutations can also be studied in their natural context on the chromosome by replacing wild-type *Cori* sequences. Surprisingly, although *Cori*-plasmids are dramatically crippled by combinations of multiple mutant CtrA binding sites, it is possible to recover viable mutants with just a single unaltered CtrA binding site remaining on the chromosome replication origin. Notably, mutations of CtrA binding site 'e' seem to be dominant and most detrimental, and other work from our laboratory has identified that this region of the origin is the most conserved among related *Caulobacter* species. All mutant strains constructed to have *Cori* with a mutation in binding site 'e' show a pronounced growth defect in M2 minimal media, are out-competed by wild-type *C. crescentus* in rich media, and do not tolerate over-supply of CtrA. The close proximity of binding site 'e' to that of the replication initator protein, DnaA, suggests that these defects may reflect a requirement for site 'e'-bound CtrA to cooperate with DnaA to potentiate replication initiation.

# A64

## IDENTIFICATION AND CHARACTERIZATION OF PROTEINS INVOLVED IN *MYXOCOCCUS XANTHUS* SPORULATION

***F. K. Tengra**[1], D. Dutton[2], J. L. Dahl[2], A. G. Garza[1];*
*[1]Syracuse University, Syracuse, NY, [2]Washington State University, Pullman, WA*

During the *Myxococcus xanthus* developmental cycle, vegetative cells differentiate into dormant, stress-resistant spores. *M. xanthus* spores are structurally complex, with several compartments that are visible using electron microscopy. The inner most compartment of the spore is the core, which contains the *M. xanthus* genome. The core is surrounded by an inner and an outer membrane, followed by a thick layer of peptidoglycan, the cortex. The cortex is surrounded by a coat which is predominantly composed of proteins. We are interested in understanding how the vegetative cell wall is restructured during sporulation and how spores acquire stress resistance. To date, only a few proteins that are directly involved in *M. xanthus* sporulation have been identified. We have used sequence homology, proteomics, and DNA microarray analysis as tools to identify additional sporulation genes/proteins. Mutations in two of the genes that we identified have morphological defects in the outer layers of the spore, the cortex and coat. These two mutants also show increased sensitivity to heat and chemical stress compared with their wild type counterparts. We are currently using mutational analysis to characterize the remaining genes that we uncovered.

# A65

## A SMALL RNA REGULATES INITIATION OF DNA REPLICATION IN *CAULOBACTER CRESCENTUS*

***L. Cheng**, L. Li, K. C. Keiler;*
*The Pennsylvania State University, University Park, PA.*

SsrA, or tmRNA, is a small RNA that interacts with selected translating ribosomes to target the nascent polypeptides for degradation. This reaction is important for both protein quality control and gene regulation in all bacteria. Deletion of the *ssrA* gene in *Caulobacter crescentus* results in a specific delay in the cell cycle before the initiation of DNA replication, but the relative

timing of subsequent events is the same as in wild type. At least three steps are required for correct timing of DNA replication in *Caulobacter*: 1) degradation of the replication repressor CtrA, 2) binding of the replication initiator DnaA to the origin of replication, and 3) transcription from a strong promoter (Ps) in the origin of replication (*Cori*). We demonstrated that the degradation of CtrA is not affected by the *ssrA* deletion. However, mass spectrometry data indicate that DnaA is tagged by SsrA at two specific sites, one close to the C terminus and the other in the DNA binding domain. Moreover, deletion of *ssrA* disrupts the timing of *dnaA* transcription, protein synthesis and degradation. Microarray assays show that, in contrast to *dnaA* transcription in wild-type *Caulobacter*, *dnaA* mRNA levels in the *ssrA* deletion strain do not increase until after replication has initiated. Likewise, pulse-labeling assays show that synthesis of DnaA protein is significantly delayed in the *ssrA* deletion strain. In addition to the disruption in *dnaA* expression, DnaA protein is more stable in the *ssrA* deletion strain, with a half-life of 80 min compared to 25 min in wild type. Because DnaA regulates its own expression, it is possible that SsrA tagging directly affects DnaA stability. To test this hypothesis, mutations in DnaA that alter its interaction with SsrA will be examined. In addition to the effects on DnaA, SsrA also alters the timing of Ps transcription. In wild-type *Caulobacter*, the Ps transcription begins after CtrA degradation, reaches a peak just before DNA replication initiation, and then decreases after initiation. In the *ssrA* deletion strain, the peak of Ps transcription is delayed by about 30 min, similar to the delay of DNA replication initiation. These data indicate that there is an SsrA-dependent, CtrA-independent factor that is required for the correct timing of Ps transcription. Together, our results indicate that SsrA is required for the correct timing of multiple steps in the initiation of DNA replication.

# A66

## THE ROLE OF THE NOVEL PROTEIN FRZS IN THE SOCIAL SWARMING BEHAVIOR OF *MYXOCOCCUS XANTHUS*

***J. P. Merlie, Jr.****, T. Mignot, D. Zusman;*
*University of California, Berkeley, CA*

The molecular mechanisms by which socially swarming bacteria coordinate the movement of individual cells within swarms remain poorly understood. During vegetative growth, groups of *Myxococcus xanthus* cells feed by coordinately moving across solid surfaces toward nutrient sources. The locomotive force for the social motility of these cells is provided for by the extrusion and retraction of the type IV pilus (TFP). Directed movement by *M. xanthus*, toward or away from attractants or repellents also requires the modulation of cell reversal frequency by a chemotaxis-like signal transduction pathway termed the Frz system. This pathway may affect reversal frequencies via effects on the biochemical activity of the TFP. However, no direct link between the Frz system and this motor has been definitively demonstrated. Previously, our lab identified a novel protein, FrzS, which may transduce signals from the core Frz pathway to the TFP. Mutations in *frzS* cause severe colony level defects in vegetative social motility. Furthermore, FrzS dynamically localizes to the cell poles - the sites of TFP assembly in *M. xanthus* cells - in a Frz dependant manner that correlates with cellular reversals (please see abstract by Mignot *et al.*). However, FrzS is not required for TFP extension or retraction in bulk cell assays. In fact, in a single cell level assay system designed to recapitulate social motility behavior FrzS appears to be dispensable for TFP based locomotion and Frz induced reversals. Why then is FrzS essential for motility at the level of the social swarm? We hypothesize that FrzS mediates the regulation of TFP activity by the Frz system specifically in the context of multicellular movement. We are now developing a method for analyzing the motility of single cells present in social swarms. Using this assay we will determine the role of FrzS in coordinating TFP based, and Frz system regulated, social motility.

# A67

## GAS VESICLES: OLD BUOYS IN NOVEL HABITATS

***G. Van Keulen***[1]*, D. A. Hopwood*[1]*, L. Dijkhuizen*[2]*, R. G. Sawers*[1]*;*
[1]*John Innes Centre, Norwich, UNITED KINGDOM,* [2]*Microbial Physiology, Groningen Biomolecular Sciences Institute (GBB), Groningen, THE NETHERLANDS*

Gas vesicles are hollow, gas-filled prokaryotic organelles that act as flotation devices. For example, they enable planktonic cyanobacteria and halophilic archaea to position themselves within the water column to make optimal use of the available light and nutrients. Very few terrestrial microbes were known to contain gas vesicles, but genome sequences are now revealing gas vesicle gene clusters in numerous bacteria from non-planktonic habitats. Examples from soils and sediments are the actinomycetes *Streptomyces*, *Frankia*, and *Rhodococcus*, including plant and animal pathogens and plant symbionts. Often, more than one set of paralogous genes is found, with two novel *gvp* genes, *gvpX* and *gvpY*, associated. For example, the *Streptomyces* model organism *S. coelicolor* has two sets of *gvp* gene clusters. Remarkably, when compared to typical GvpA sequences, the major gas vesicle protein GvpA of actinomycetes has a long C-terminal extension containing unusually high amounts of arginine, glutamate and proline residues. An intriguing question, therefore, is to determine what the physiological function(s) of these *gvp* gene clusters in these complex soil organisms might be. An *S. coelicolor gvp* knockout strain, with both *gvp* gene clusters deleted, could still float in standing liquid cultures, suggesting an alternative function for these gas vesicles in actinomycetes. We also noted, however, that the *gvp* mutant strain showed a severe aerial developmental phenotype on certain solid growth media. The possible roles of Gvp proteins in *S. coelicolor* differentiation will be discussed.

# A68

## BIOCHEMICAL AND GENETIC IDENTIFICATION OF A C-DI-GMP BINDING MOTIF

***B. Christen**, M. Christen, U. Jenal;*
*University of Basel, Basel, SWITZERLAND*

Cyclic di-guanosine monophosphate (c-di-GMP) has recently been shown to be a novel secondary messenger involved in regulating bacterial motility and community behavior. An increasing number of genetic studies in different bacteria showed that c-di-GMP controls the developmental switch between a motile, planktonic and a surface attached biofilm mode. Diguanylate cyclases (DGC), the enzymes that catalyze the formation of c-di-GMP from GTP, are widespread in bacteria and the presence of a large number of paralogs in many bacterial species poses the question of how DGCs from parallel signal transduction pathways are controlled.
We use *Caulobacter crescentus* PleD, a multi domain response regulator protein with a DGC (GGDEF) output domain as a model to investigate controlled synthesis of c-di-GMP in bacterial cells. We have found that the diguanylate cyclase activity of PleD is activated in response to phosphorylation but in addition is subject to direct feedback inhibition by its own product. The recently solved crystal structure of PleD shows that c-di-GMP does not only bind to the active site (A-site) of the DGC domain, but also to a putative allosteric binding site (I-site). Using site-directed mutagenesis in combination with UV cross-linking and tryptic digest experiments, we confirmed the existence of the I-site in solution. Here we present evidence that an additional *C. crescentus* GGDEF protein, CC3285, exhibits DGC activity and is regulated by the same c-di-GMP feedback inhibition mechanism. To probe the nature of the I-site binding pocket, we generated a CC3285 I-site mutant library by randomized but targeted mutagenesis and used a novel genetic screen to select for highly active mutants that had lost feedback control but maintained catalytic DGC activity. The results will be discussed with respect to the ligand binding properties and the regulatory mechanisms involved in allosteric control of DGC proteins. This is the first binding motive identified for the novel secondary messenger c-di-GMP. Our results and the observation that the amino acids of the I-site pocket are conserved in a large fraction of the known GGDEF proteins, argue that allosteric product inhibition is a general control mechanisms of bacterial DGCs.

# A69

## ROLE OF *MREB* IN THE CONTROL OF CELL SHAPE IN *B. SUBTILIS*

***A. Formstone**, J. Errington;*
*Sir William Dunn School of Pathology, University of Oxford, Oxford, UNITED KINGDOM*

MreB shares a common prokaryotic ancestor with actin and is present in almost all rod-shaped bacteria. MreB proteins have been implicated in a range of important cell processes, including cell morphogenesis, chromosome segregation and cell polarity.
Construction of an in-frame deletion of *mreB* and its complementation by *mreB*$^+$ only, *in trans*, established that the gene is important for maintenance of cell width and cell viability under normal growth conditions, independent of polar effects on downstream genes. Remarkably, virtually normal growth was restored to the *mreB* null mutant in the presence of high concentrations of magnesium, especially when high concentrations of the osmoprotectant, sucrose were also present. Under these conditions, cells could be maintained in the complete absence of an *mreB* gene, with almost normal morphology. No detectable effect on chromosome segregation was evident in the mutant, nor was there an effect on the topology of nascent peptidoglycan insertion. Propagation of the *mreB* null mutant in the absence of magnesium and sucrose led to a progressive increase in cell width, culminating in cell lysis. Cell division was also perturbed but this effect may be secondary to the disturbance in cell width. These results suggest that the major role of MreB in *B. subtilis* lies in the control of cell diameter (Formstone and Errington, 2005 *Mol Microbiol* **55**: 1646-1657). *B. subtilis* contains three actin homologues MreB, Mbl and MreBH. We have begun to elucidate the structural and functional relationship between these homologues to establish their unique or redundant roles within the cell.

# A70

## METABOLOME ANALYSES OF MYXOCOCCUS XANTHUS REVEAL BIOMARKERS OF MYXOBACTERIAL DEVELOPMENT

***H. B. Bode**[1], M. Ring[1], R. M. Kroppenstedt[2], S. Schulz[3], D. Kaiser[4], G. Schwär[1];*
*[1]Saarland University, Saarbrücken, GERMANY, [2]German National Resource Centre for Biological Material (DSMZ), Braunschweig, GERMANY, [3]Technical University Braunschweig, Braunschweig, GERMANY, [4]Stanford University, Stanford, CA*

With their complex life cycle culminating in the formation of myxospores in sophisticated fruiting bodies (1), myxobacteria are an ideal model to investigate the physiological changes occurring during the underlying processes. We have focused on metabolome analyses using gas chromatography (GC) and high performance liquid chromatography (HPLC) coupled to mass chromatography (MS) in order to get a better understanding of the biochemistry behind myxobacterial fruiting body formation and sporulation. Especially GC/MS has a proven track record as a powerful tool to analyze complex biological samples consisting of several low molecular weight compounds such as primary and intermediary

metabolites (i.e. amino acids, sugars, lipids). Using this technique we were able to identify known biomarkers of myxobacterial development (accumulation of the A-signal mixture of amino acids (2) during early development and trehalose in the spores (3)). We have also identified previously unknown biomarkers. A specific increase during development of two unusual etherlipids and a fatty acid aldehyde were observed and shown to be spore-specific (a 40-fold increase was observed in the spores compared to vegetative cells). Analysis of several fruiting body-deficient mutants revealed a strongly reduced amount of these lipids in esg (E-signal defective (4)) mutants that have a reduced amount of isovaleryl-CoA (5,6). Isovaleryl-CoA is the starting unit for iso-fatty acids, the dominant fatty acid family in myxobacteria (7). Furthermore, esg mutants were rescued by the addition of isovaleric acid and the corresponding fatty acid and lipid derivatives derived thereof. Due to the high amount of the lipid compounds produced during development we suggest their major function to be structural to protect myxospores from hydrolysis. However, an additional signaling function could not be excluded. Finally we tested if mutants depleted in the amount of non-iso fatty acids (the second important fatty acid family in M. xanthus), show developmental defects as well. Whereas no difference between such mutants and the wildtype could be observed, reduction of the amount of iso-fatty acids resulted in severe developmental defects (see above) furthermore indicating the important role of iso-fatty acids for myxobacteria.

1. Kaiser, D. (2004) Annu Rev Microbiol 58, 75-98
2. Kuspa, A. et al. (1992) J Bacteriol 174, 3319-3326
3. McBride, M. J., and Zusman, D. R. (1989) J Bacteriol 171, 6383-6386
4. Toal, D. R. et al. (1995) Mol Microbiol 16, 177-189
5. Mahmud, T., Bode, H. B., Silakowski, B., Kroppenstedt, R. M., Xu, M., Nordhoff, S., Höfle, G., and Müller, R. (2002) J Biol Chem 277, 23768-32774
6. Mahmud, T., Wenzel, S. C., Wan, E., Wen, K. W., Bode, H. B., Gaitatzis, N., and Müller, R. (2005) ChemBiochem 6, 322-330
7. Bode, H., Dickschat, J. S., Kroppenstedt, R. M., Schulz, S., and Müller, R. (2005) J Am Chem Soc 127, 532-533

# A71

## A NOVEL PROTEIN MEDIATES THE CONTROLLED DEGRADATION OF A CELL CYCLE MASTER REGULATOR AT THE DYNAMICALLY LOCALIZED CLPXP PROTEASE

***P. T. McGrath***[1]*, K. Ryan*[2]*, A. Iniesta*[1]*, L. Shapiro*[1]*, H. McAdams*[1]*;*
*[1]Stanford University, Stanford, CA, [2]University of California, Berkeley, Berkeley, CA*

We are exploring the genetic circuitry controlling the multiple modules involved in the execution of the *Caulobacter crescentus* cell cycle. In addition to the contributions of transcriptional control networks and phosphorylation cascades, protein localization and targeted proteolysis play a key role in controlling cell cycle progression. The multimeric ClpXP proteolytic complex, comprised of the ClpX ATPase and the ClpP protease, is responsible for regulated protein degradation required for cell cycle progression in *Caulobacter*. One critical function of ClpXP is to clear the CtrA master regulator of the cell cycle from the cell quickly at specific times to allow the G1-S transition. We report here that this protease complex is dynamically localized to specific cellular sites as a function of the *Caulobacter* cell cycle. Several ClpXP substrates, including CtrA, are co-localized at the cell pole with ClpXP at the time of their specific proteolysis, and this co-localization is required for their proteolysis. ClpXP transiently appears at the division plane in late pre-divisional cells, and we hypothesize that an additional set of unidentified ClpXP substrates are degraded at this place and time. We also took a bioinformatics approach to identify an adaptor protein, RcdA, that mediates the interaction between CtrA and the ClpXP protease. RcdA is required for both the polar localization and proteolysis of CtrA, and is itself transiently localized to the cell pole and the division plane coincident with and dependent upon ClpX localization.

# A72

## THE PLED DIGUANYLATE CYCLASE IS ACTIVATED THROUGH DIMERIZATION AND IS SUBJECT TO TIGHT ALLOSTERIC FEEDBACK CONTROL

***R. Paul****, M. Christen, B. Christen, A. Schauerte, P. Jeno, U. Jenal; University of Basel, Basel, SWITZERLAND*

The response regulator PleD is required for pole development during the *Caulobacter crescentus* life cycle. PleD contains two N-terminal receiver domains and a C-terminal diguanylate cyclase (DGC) domain (Paul et al. 2004). Upon phosphorylation of the first receiver domain by its cognate kinase, the DGC readout is activated and two molecules of GTP are converted into the secondary messenger c-di-GMP. The resolution of the PleD crystal structure not only suggested that phosphorylation of PleD might result in its activation by dimerization, but had also proposed that the DGC activity is under tight negative allosteric control (Chan et al. 2004). Here we present evidence that in vitro activation of PleD with beryllium fluoride resulted in a strong increase of DGC activity and an enhanced tendency for dimer formation. Covalently cross-linked PleD dimers showed a highly increased enzymatic activity compared to non-crosslinked PleD monomers. PleD variants with mutations in the proposed dimer interphase could not be activated by beryllium fluoride and showed no dimerization. Together, this suggests that the enzymatically active form of PleD is a dimer, and that phosphorylation induces PleD dimer formation.
We also probed the proposed allosteric regulatory site of PleD (I-site) using a specific binding assay with radiolabeled c-di-

GMP. Protease protection studies confirmed that c-di-GMP binds to the I-site in solution. The I-site pocket is formed by specific residues in receiver domain 2 and in the C-terminal DGC domain. Replacing the I-site residues R359, D362 and R390 (all contained within the DGC domain) with alanine resulted in the complete loss of c-di-GMP binding. In contrast, mutation of residues R148 and R178 (located in the Rec2 domain) did not affect I-site binding of c-di-GMP. Together this argued that the I-site and allosteric inhibition is fully contained within the C-terminal DGC domain. While I-site mutations in the DGC domain abolished or strongly reduced c-di-GMP formation, I-site mutations in the Rec2 domain resulted in an increased enzymatic activity. This finding indicates a possible role of the Rec2 domain in modulating PleD activity and will be discussed with respect of the unusual domain architecture of this response regulator. Based on these results we propose that diguanylate cyclase activity of PleD is controlled by two general mechanisms, phosphorylation-induced dimerization and allosteric feedback inhibition.

Paul, R., S. Weiser, et al. (2004). Cell cycle-dependent dynamic localization of a bacterial response regulator with a novel di-guanylate cyclase output domain. Genes Dev **18**(6): 715-7

Chan, C., R. Paul, et al. (2004). Structural basis of activity and allosteric control of diguanylate cyclase. Proc Natl Acad Sci U S A **101**(49): 17084-9

# A73

## FLIX AND FLBD OF *CAULOBACTER CRESCENTUS* FORM STABLE *IN VIVO* COMPLEX, WHICH IS REQUIRED FOR THEIR REGULATORY ACTIVITIES

***J. W. Gober****, Z. Xu;*
*UCLA, Los Angeles, CA*

The temporal and spatial expression of late flagellar genes in *Caulobacter crescentus* is activated by the transcription factor FlbD and its partner trans-acting factor, FliX, which functions as both a positive and negative regulator of FlbD activity, probably via direct interaction. Both FlbD and FliX are conserved in alpha-proteobacteria possessing polar flagellum. Structure prediction of FliX revealed that wild type FliX forms two coiled-coil structures, one located in the conserved central and another in the carboxyl-terminal regions. In this study, affinity chromatography and co-immunoprecipation experiments were performed to investigate the relationships between FlbD and FliX interaction and their regulatory activities. Our data demonstrated that FliX coupled to sepharose beads could immobilize FlbD from crude cell extract even under salt concentration up to 2.65M, indicating a remarkably high affinity between FliX and FlbD. In addition, immunoprecipitation experiments demonstrated that FliX can be co-precipitated with FlbD by anti-FlbD antibodies, suggesting the existence of an *in vivo* complex of the two proteins. A site-directed mutation of FliX that is predicted to abolish the conserved central coiled-coil region, L85K, resulted in a mutant protein failed to interact with FlbD. Another FliX mutant, Δ117-8, in which the distance between the two coiled-coil regions was shortened, demonstrated a dramatically reduced ability to bind FlbD. Other FliX mutants, R71A, T130L, L136K and FliX1, whose secondary structures were not predicted to be altered significantly, maintained their strong association with FlbD. Previous gene expression studies also demonstrated a completely diminished activity of L85K and a reduced activity of Δ117-8 to regulate the transcription of class II and class III/IV promoters, reflecting an intrinsic connection between FliX structure, FliX/FlbD interaction, and their regulatory functions. In support of this idea, two FlbD alleles, FlbD-1204 and FlbD-1231 were also examined for their ability to bind FliX. Despite the fact that wild type FlbD and the two FlbD mutants are expressed at the same level in *Caulobacter* cells, the FlbD mutants demonstrated an enhanced binding capacity to FliX, a property that might contribute to their ability to transcribe class III/IV flagellar genes without the completion of early basal body structure composed of class II structural proteins. Based on these results, we conclude that FlbD and FliX form stable *in vivo* complex, and any alteration of the stability of the complex results in abnormal functionality of the two proteins.

# A74

## *STREPTOMYCES VENEZUELAE*: A STREPTOMYCETE THAT SPORULATES IN SUBMERGED LIQUID CULTURE

***Maureen J. Bibb****[1], Mervyn J. Bibb[1], Mark J. Buttner[1], Govind Chandra[1], Rekha Chakraburtty[2], Brian Green[2], Eric Mathur[2], Ashish Paradkar[2], Jamie Ryding[2], Jay Short[2], Roy Williams[2]*
*Department of Molecular Microbiology, John Innes Centre, Norwich, UK and [2]Diversa Corporation, San Diego, CA*

In the extensively studied model species *Streptomyces coelicolor* differentiation occurs only on solid medium. In contrast, *Streptomyces venezuelae* sporulates to completion in liquid culture, and thus provides an unusual opportunity to study developmental gene expression in synchronously growing cultures. *Streptomyces diversa*™ is a derivative of *S. venezuelae* that has been genetically engineered for enhanced secondary metabolism. The availability of the *S. diversa*™ genome sequence has enabled us to advance *S. venezuelae* as a developmental system. We have constructed a minimal, ordered cosmid library, currently covering 83% of the chromosome, allowing us to apply the rapid, PCR-targeted "Redirect" gene disruption methodology of Gust *et al.* (2003) to this species. Through this route, we have begun to create null mutations in the *S. venezuelae* orthologues of the known developmental genes of *S. coelicolor.* The phenotypes of these null mutants, revealed by phase contrast and scanning electron microscopy, will be presented and compared to the phenotypes of the equivalent *S. coelicolor* mutants. Ultimately, *S. venezuelae* and its developmental mutant derivatives will allow detailed transcriptional profiling of *Streptomyces* sporulation by microarray analysis.

# A75

## EPISTATIC RELATIONSHIPS OF TWO REGULATORY FACTORS DURING HETEROCYST DEVELOPMENT

***Ho-Sung Yoon**** *and James W. Golden*
*Department of Biology, Kyungpook National University, Daegu, South Korea Department of Biology, Texas A&M University, College Station, Texas, USA*

The filamentous cyanobacterium *Anabaena* sp. Strain PCC 7120 produces a developmental pattern of single heterocysts separated by approximately 10 vegetative cells. Heterocysts differentiate from vegetative cells and are specialized for nitrogen fixation. The *patS* gene, which encodes a small peptide that inhibits heterocyst differentiation, is expressed in proheterocysts and plays a critical role in establishing the heterocyst pattern. A *patS-gfp* reporter strain revealed clusters of *patS*-expressing cells during the early stage of heterocyst differentiation. PatS signaling is likely to be involved in the resolution of these clusters. Another key regulator of heterocyst development is the *het*R gene. *het*R mutants fail to produce heterocysts and extra copies of *het*R on a plasmid cause a multiple contiguous heterocyst phenotype. To elucidate the relationship between the two counter acting factors, *patS* and *het*R, in the genetic regulatory pathway during heterocyst differentiation, the expression patterns of a *patS-gfp* and a *hetR-gfp* fusion were examined in a *patS* deletion and a *het*R deletion strain. The results, in combination with the result from a *het*R and *patS* double deletion strain, suggest *patS* and *het*R are mutually antagonistic and the balance between these two factors in two different cell types (heterocysts and vegetative cells) may be critical during the decision making process on their cell fates.

# A76

## CHARACTERIZATION OF AN ECF SIGMA FACTOR OPERON THAT REGULATES EARLY *MYXOCOCCUS XANTHUS* DEVELOPMENTAL GENE EXPRESSION

*M. Esmaeiliyan, J. J. Rivera, and* ***H. B. Kaplan***
*Univ. Texas Medical School, Houston, TX*

*Myxococcus xanthus* is a Gram-negative soil bacterium that undergoes multicellular development upon starvation at high cell density. Expression of the early developmental gene *4445* requires starvation, high cell density, aeration, and at least 8 mM $Mg^{++}$. Random mutagenesis with the Himar1-tet transposon and subsequent genetic experiments identified the regulators of *4445* expression to be a positive regulator, EcfA, and two negative regulators, ReaA and ReaB. This *M. xanthus* EcfA/ReaA/ReaB signal transduction system is encoded by one operon and is analogous to the *E. coli* sigmaE/RseA/RseB signal transduction system that is activated by certain envelope stresses.

Protein localization and topology of ReaA and ReaB were examined by constructing alkaline phosphatase (PhoA) fusions and testing their activity in *E. coli*. ReaA appears to have a central transmembrane domain, a cytoplasmic N-terminus and a periplasmic C-terminus. ReaB appears to have a signal peptide sequence that transports the protein to the periplasm. These data suggest that ReaA and ReaB are functional homologues of *E. coli* RseA and RseB.

Mutants containing *reaA* or *reaB* mutations express *4445* at levels more than three orders of magnitude greater than wild-type cells during growth and development. These same mutants overproduce EcfA during growth and development indicating that the *ecfA* operon is autoregulated. The expression of *4445* is increased three fold during growth by the addition of 0.5 M glycerol, which induces starvation-independent sporulation. In addition, LPS O-antigen mutations bypass the high-density requirement for *4445* expression during starvation in an *ecfA*-dependent manner. These data indicate that the *ecfA* operon encodes the elements of a new regulatory pathway that integrates and transduces starvation and cell density cues during early *M. xanthus* development and also senses cell surface alterations.

# A77

## MEASUREMENT OF BACTERIAL ADHESION FORCE BY MICROMANIPULATION

***J.X. Tang[1],*** *G. Li[1], Y. V. Brun[2], P. Tsang[1];*
*[1]Brown University, Providence, RI, [2]Indiana University, Bloomington, IN*

*Caulobacter crescentus* has a dimorphic life cycle, and has been extensively studied as a model system for prokaryotic development. Following the growth cycle, the free swimming swarmer cell shed its flagellum and grows a stalk with a holdfast, which can attach to a solid surface. The adhesive property probably provides an advantage for the cell to remain in a region of nutrients availability. Using a micromanipulation technique, we measured the adhesion force of single *Caulobacter crescentus* stalked cells attached to borosilicate substrates through their adhesive holdfast. The detachment force ranges over 0.11 to 2.26 μN for 14 cells, and is nominally averaged 0.59±0.62 μN. This is by far the strongest force of adhesion measured for bacterial cells. The size of the holdfast is on the order of 100 nm in diameter, measured by imaging using an atomic force microscope. Therefore, the strength of the holdfast adhesion is estimated to be as high as 60 N/ $mm^2$. Such strength of adhesion is the strongest ever measured for biological adhesives.

# B1

## CELL CYCLE-REGULATED PROTEOLYSIS OF PODJ, A POLARITY DETERMINANT IN *CAULOBACTER CRESCENTUS*

***J. C. Chen***[1], *P. H. Viollier*[2], *P. T. McGrath*[1], *A. K. Hottes*[1], *H. H. McAdams*[1], *L. Shapiro*[1];
[1]*Stanford University, Stanford, CA,* [2]*Case Western Reserve University, Cleveland, OH*

*Caulobacter crescentus* cells undergo specific morphological changes as they progress through the cell cycle. The polarity determinant PodJ plays a critical role in these changes by recruiting structural and regulatory proteins needed for organelle biogenesis to the correct cell pole at defined times during the cycle. Regulated, sequential proteolysis contributes to the asymmetric localization of PodJ to the appropriate pole. Full-length PodJ ($PodJ_L$) is synthesized early in the cell cycle and truncated to a shorter form ($PodJ_S$) around the time of cell division; $PodJ_S$ is then cleared from the cell in the ensuing cycle, before $PodJ_L$ is re-synthesized. We have determined that a functionally conserved membrane metalloprotease (MmpA) participates in the clearance of $PodJ_S$. MmpA appears to cleave within the single transmembrane segment of $PodJ_S$, releasing it into the cytoplasm for complete proteolysis. In addition, we have identified the putative protease (PerP) that converts $PodJ_L$ to $PodJ_S$. The expression of PerP is regulated by a signal transduction cascade (the DivJ-PleC-DivK pathway) that activates cell-specific transcription programs in response to cytokinesis. This coupling ensures that truncation of $PodJ_L$ to $PodJ_S$ occurs only after division is completed and the two daughter cells are compartmentalized. Constitutive expression of PerP prevents the accumulation of $PodJ_L$ and disrupts the developmental events that it controls. Since PleC depends on PodJ for correct localization, PodJ, PleC, and PerP constitute the components of a regulatory feedback loop: PodJ recruits PleC to the correct cell pole, appropriately localized PleC allows PerP expression after division, and PerP converts $PodJ_L$ to $PodJ_S$.

# B2

## INHIBITION OF CELL DIVISION BY SULA IS ESSENTIAL FOR PERSISTENCE OF UROPATHOGENIC ESCHERICHIA COLI IN THE BLADDER

***S. S. Justice***, *P. C. Seed, D. A. Hunstad, S. R. Lauer, J. N. Walker, S. J. Hultgren;
Washington University, St. Louis, MO*

During an acute episode of cystitis, uropathogenic Escherichia coli (UPEC) form intracellular bacterial communities (IBCs) within the superficial umbrella cells of the bladder. The bacteria within the IBCs undergo 3 unique stages of differentiation in a process that resembles the maturation of bacteria grown on abiotic surfaces. Inhibition of cell division of a small subset of bacteria results in the formation of filamentous UPEC up to 70 μm in length. Filamentation appears to be in response to a TLR-4-mediated innate defense mechanism. Surface filaments appear to survive the infiltrating polymorphonuclear leukocytes (PMNs), suggesting filamentation may play a role in pathogenesis. In order to directly assess a role for filamentation during acute cystitis, mutants defective in inhibition of cell division were investigated. Filamentation of UPEC in the bladder appears to require the cell division inhibitor component of the SOS response, SulA. The SOS response is a group of at least 17 genes that are responsible for repair of damaged DNA. Inactivation of sulA led to a severely attenuated UPEC strain. The sulA strain was defective in maintaining an acute infection and was unable to develop a persistent infection in the wild type mouse model. In contrast, the mutant was fully virulent in the isogenic TLR-4 deficient mouse. As filamentation does not occur during the acute infection in the TLR-4 deficient mouse strain, this data supports the hypothesis that filamentation is required for evasion of the host immune response consequently leading to the establishment of a persistent infection. Furthermore, a reporter for transcription from PsulA revealed the differential expression of sulA within the IBCs of the mouse bladder. It is yet unclear whether the expression of sulA is a consequence of the classical SOS response or due to another mechanism. In addition, it the molecular mechanism(s) for evasion of the PMNs has yet to be elucidated. However, filamentation alone is not sufficient to promote survival of UPEC in the presence of activated PMNs in vitro. Further characterization of the interaction between bacterial filaments and the immune response could lead to better therapeutics.

# B3

## A NOVEL MECHANISM FOR DIVISION PLANE LOCALIZATION IN *CAULOBACTER*

***M. Thanbichler***, *L. Shapiro;
Stanford University, Stanford, CA*

Correct positioning of the division septum is a prerequisite for successful cell division. In many bacteria, this process is regulated by oscillatory waves of MinCDE that inhibit FtsZ localization at the poles of the cell and threreby direct the division machinery toward midcell. However, a number of organisms lack homologs of these proteins. Here, we present a novel mechanism that couples assembly and positioning of the the Z-ring to the initiation of chromosome replication and bipolar localization of the duplicated origin regions in *C. crescentus*. It is based on the activity of a newly identified essential protein, named MipZ, which forms a dynamic complex with the putative partitioning protein ParB close to the origin of replication. At the beginning of the cell cycle, the origin is located at the stalked pole of the cell, while FtsZ

forms a focus at the opposite pole, derived from the previous septation process. Initiation of DNA replication generates two daughter origins, both of which become bound by the ParB-MipZ complex. One of the copies remains at the starting position, and the second traverses the cell to adopt its position at the pole occupied by FtsZ. As soon as the origin-ParB-MipZ complex arrives, FtsZ is dissipated and forms a ring positioned in-between the two origin regions. Depletion of MipZ results in aberrant localization of FtsZ polymers in the cell. By contrast, overproduction of MipZ causes immediate disassembly of existing FtsZ rings, thereby leading to cell filamentation. Taken together, these observations indicate an inhibitory effect of MipZ on FtsZ polymerization, which prevents Z-ring formation close to the cell poles and initiation of cell division before duplication of the origins has occured.

# B4

## INVESTIGATION OF THE BIOLOGICAL FUNCTION OF THE ESAT-6 GENE CLUSTER OF *STREPTOMYCES COELICOLOR*

***s. akpe san roman;***
*University of Wales swansea, Swansea, UNITED KINGDOM*

The biological function of the *ESAT-6* gene cluster is unknown, the cluster is located in RD1, a 10 kb DNA region deleted in the attenuated tuberculosis vaccine strain Mycobacterium bovis BCC. It encodes for the early secreted antigenic target 6 kDa protein (ESAT-6) and culture filtrate protein of 10 kDa (CFP-10) both potent T-cell antigens among other proteins involved in energy producing processes. Current research provided important links of the cluster with virulence in *Mycobacterium tuberculosis*, proposing that the *ESAT-6* genes work together to form a single virulence determinant, and argue that cluster encodes a novel specialized secretion system that is required for pathogenesis (Guinn *et al.* 2004). Other studies point out the ***ESAT-6*** locus regulates DNA transfer in *M. smegmatis* (Flint *et al.* 2004). We used in vitro transposon mutagenesis to obtain Tn*5062* insertions in cosmid SC3C3 that contains the *ESAT-6* cluster. We obtained mutants of genes *SCO5724 cfp-10* like and *SCO5725 esat-6* like. Phenotypic examination of the mutants has revealed that they are delayed in morphological differentiation. We showed that the products of these genes interact *in vitro* like their counterparts in *Mycobacterium* and localisation studies suggest that SCO5724 is secreted in the culture medium. Further studies will determine which components of the cluster are essential for SCO5724 and/or SCO5725 secretion.

# B5

## DEFINING THE V. HARVEYI QUORUM SENSING REGULON: LUXR, THE MASTER REGULATOR

***A. Pompeani****, B. L. Bassler;*
*Princeton University, Princeton, NJ*

Quorum sensing is a process of cell-cell communication that allows bacteria to measure their population density by monitoring the levels of small, secreted signal molecules called autoinducers. The model bacterium *Vibrio harveyi* uses at least two quorum sensing systems, facilitating both intra- and inter-species communication. System 1, responsible for intra-species communication, is composed of LuxN, which binds autoinducer-1 (AI-1; N-(3-hydroxybutanoyl)-L-homoserine lactone). System 2 provides inter-species communication and is composed of a periplasmic binding protein, LuxP, which binds autoinducer-2 (AI-2; a furanosyl borate diester), and interacts with LuxQ to transduce AI-2 information. Sensory information from both systems impinges on LuxR, the master quorum sensing regulator. In *V. harveyi*, this circuit controls the expression of genes responsible for many processes, including bioluminescence, type-III secretion, biofilm formation, and protease production. Our data indicate that LuxR controls all of the members of the quorum sensing regulon. To identify promoters directly regulated by LuxR, fluorescence activated cell sorting (FACS) is being used to screen *E. coli* containing *luxR* and random *V. harveyi* promoters fused to *gfp*. Three targets have been identified, and include a putative thioester hydrolase protein, a predicted nucleoside-diphosphate-sugar epimerase, and a putative drug and metabolite transporter. Future research will focus on characterizing the LuxR-controlled gene regulation hierarchy by analyzing the identified promoters, their affinites for LuxR, and the dynamics of their responses to autoinducers.

# B6

## ENHANCED REPAIR OF DNA DAMAGE MAY PROVIDE THE EVOLUTIONARY DRIVE FOR BIOLUMINESCENCE IN THE INSECT PATHOGEN *PHOTORHABDUS LUMINESCENS*

***V. Salisbury****, K. Cutter;*
*University of the West of England, Bristol, UNITED KINGDOM*

Bioluminescent bacteria are common in marine environments but only a single terrestrial example, *Photorhabdus luminescens*, has been characterised. Light is produced in these bacteria by a luciferase enzyme, coded by *luxAB*, which is stable at 37$^{o}$C, and a fatty acid reductase, encoded by *luxCDE*, required for provision of the aldehyde substrate. *P. luminescens luxCDABE*

genes have been ligated into broad host range plasmids, downstream of constitutive promoters, and expressed in a variety of bacteria, to give self-bioluminescent reporter strains. Bacteria transformed with plasmids carrying the *luxAB* genes require the addition of exogenous decanal substrate for light production. *E.coli* DH5α transformed with pAL2 (*luxCDABE*) and pAL1 (*luxAB*) were exposed to UV light followed by incubation in the dark. The self bioluminescent pAL2 transformants survived significantly better than the non-light emitting pAL1 transformants, indicating that the light production, activating DNA repair by photolyase, plays a significant role in surviving the effects of DNA damaging agents. It has also been observed that in both wild type *P. luminescens* and *E. coli lux*+ constructs, cultures treated with low levels of DNA intercalating agents or DNA gyrase inhibitors show increased light output (between 5 and 10 fold), compared to untreated controls, This suggests that both sub-lethal DNA damage and changes in DNA supercoiling lead to promoter independent up-regulation of luminescence in wild type *P. luminescens* and self-bioluminescent constructs of a range of bacteria expressing *luxCDABE*. The results are in agreement with previous studies showing that *lux* genes from marine bacteria are up-regulated as part of the general stress response in prokaryotes and may function to provide enough visible light for photoreactivation. With regard to *P.luminescens*, where the insect host is thought to release a variety of defensins in response to infection, light output may lead to increased survival and therefore increased virulence of these prokaryotic insect pathogens.

# B7

## BIOCHEMICAL ANALYSIS OF A FTSZ MUTANT DEFICIENT IN THE INITIATION AND IN LATE STAGES OF CELL DIVISION IN THE BACTERIUM *CAULOBACTER CRESCENTUS*

***M. J. Trimble****, Y. Wang, Y. V. Brun;*
*Indiana University, Bloomington, IN*

*Caulobacter crescentus* and other prokaryotes use many proteins in cell division. FtsZ, a tubulin-like GTPase, is the most conserved protein involved in bacterial cell division and is believed to act at its earliest step. Upon binding GTP, FtsZ oligomerizes and forms a ring known as the Z-ring around the circumference of the mid-cell. GTP hydrolysis by FtsZ is thought to drive the constriction of the cell, although the mechanism of constriction is not known. We have been studying a FtsZ mutant that appears to be deficient in early and late stages of cell division. Two charged residues at positions 142 and 144 were mutated to alanines, yielding FtsZEGR142AGA (FtsZ142). Sequence similarity to *Escherichia coli* FtsZ indicates these residues are involved in ribose binding. The phenotype of the mutant was analyzed by expressing it in a wild-type FtsZ depletion strain. After depletion of wild-type FtsZ, cells expressing FtsZ142 exhibit a temperature-sensitive phenotype with long unconstricted polar areas and extended areas of deep constriction at the mid-cell when grown at 22°C. Immunolocalization studies showed that FtsZ is present in both the constricted and the unconstricted regions. To study the mutant protein *in vitro*, FtsZ142 was overexpressed in *E. coli* and its purity was determined by mass spectrometry. Size exclusion chromatography indicates that FtsZ142 behaves as a multimer greater than 1500 kDa. The multimeric nature of overexpressed FtsZ142 was also observed by native gel chromatography. FtsZ142 binds GTP and has GTPase activity greater than wild-type. These phenotypic and biochemical analyses of FtsZ142 suggests a model where FtsZ142 is able to form Z-rings and constrict, perhaps after wild-type FtsZ has intiated the process, but is unable to depolymerize to complete cell division.

# B8

## AN ANTI-SIGMA FACTOR WITH NOVEL CHARACTERISTICS IN *STREPTOMYCES COELICOLOR A3(2)*

***A. A. Gaskell****, G. Kelemen;*
*UEA, Norwich, UNITED KINGDOM*

*Streptomyces coelicolor* is a model organism for both development and antibiotic production. The genome sequencing project now completed reveals a genome with nearly twice as many genes as *Escherichia coli*. RNA polymerase heterogeneity is extraordinary in *S.coelicolor*, which possesses ~63 sigma factors. Nine (SigF-N) of the sub-family of sigma factors have been identified recently, including SigF, which controls late sporulation, and SigH and SigN, which are associated with both early morphogenesis (aerial growth) and responses to specific stress conditions. In *Bacillus subtilis* $\sigma^B$ is regulated by the partner switching of RsbW, an anti-sigma factor, between $\sigma^B$ or RsbV. In *S.coelicolor* numerous (~29) anti-sigma factors homologous to RsbW have been identified, raising the question of the sigma factor-anti sigma factor promiscuity amongst this family.
We are examining the role and regulation of a member of this family, $\sigma^M$, and the anti-sigma factor, PrsM. The *sigM* mutant is more sensitive to disulphide stress suggesting a role of $\sigma^M$ in maintaining a reduced cytosolic environment in *S.coelicolor*. Similar to RsbW, PrsM possesses a HATPase_c domain expected to be involved in phosphorylation of a partner protein. In addition, PrsM has seven cystein residues that might be sites for metal coordination. Consistent with this, overexpressed and purified PrsM gave an absorption spectrum characteristic of an iron sulphur cluster.

# B9

## TRANSFORMATION AND HORIZONTAL DNA TRANSFER IN COLONY BIOFILM OF ESCHERICHIA COLI

***S. Maeda**, Y. Ishimoto, A. Matsuda, A. Sawamura, S. Kato;*
*Nara Womens' University, Nara, JAPAN*

We report that colonial Escherichia coli cells on various solid media can develop modest genetic competence. Using an on-filter culture system, we found that E. coli colonies on CaCl2-containing agar were transformed in the presence of plasmid DNA. Transformation also occurred on LB agar, various moist foods and even on H2O agar. These results suggest that some populations of colonial E. coli in various environments could become transformable regardless of the surrounding Ca2+ concentration. Furthermore, we found that simple culturing of mixed E. coli strains on LB agar in a colony biofilm can trigger in situ lateral transfer of non-conjugative plasmid without any special treatment. Liquid cultures in LB broth produced very few or no transformants, indicating the importance of biofilm formation. These results suggest that non-conjugative, non-viral horizontal gene transfer between E. coli cells can occur more easily than previously thought.

# B10

## FUNCTIONAL ANALYSIS OF SSGA, A SPORULATION-SPECIFIC ACTIVATOR OF CELL DIVISION IN STREPTOMYCETES

***B. A. Traag**, G. P. van Wezel;*
*University of Leiden, Leiden, THE NETHERLANDS*

Regulation of sporulation-specific cell division in streptomycetes is a complex process, which is only superficially understood. The sporulation process in aerial hyphae can be divided into distinct landmark events, starting with the simultaneous formation of many FtsZ rings, followed by initial septum formation, DNA segregation, and spore maturation. SsgA, a protein unique to sporulating actinomycetes, was initially discovered as an activator of submerged sporulation of *Streptomyces griseus*. *ssgA* null-mutants of *S. coelicolor* and *S. griseus* are defective in sporulation, while overexpression leads to hyperseptation and fragmentation in *S. coelicolor*. Transcription of *ssgA* is strongly enhanced during the initiation of sporulation, supporting an important role for SsgA during the later stages of the *Streptomyces* development. In *S. coelicolor* transcription is fully dependent on SsgR, an IclR-type regulator, which binds specifically to the promoter region (Traag et al. (2004), Mol. Microbiol. 53: 985-1000). This is in clear contrast with the situation in *S. griseus*, where transcription is activated by A-factor (through AdpA) and control by the SsgR orthologue is far less important. SsgA is involved in the control of sporulation and morphology in perhaps all sporulating actinomycetes. Several equally unique homologes have been identified in streptomycetes as well as other actinomycetes such as *Thermobifida* and *Kineococcus*. Besides the impact on developmental biology, the ability of SsgA to stimulate fragmented growth is highly relevant for biotechnology, and is currently exploited to improve the general growth characteristics of actinomycetes in fermentations. Present work focuses on isolation and structural analysis of SsgA whereby we hope to obtain a better understanding of the mode of action of this new class of developmental regulators. Our most recent findings will be presented.

# B11

## DEVELOPMENTAL CONTROL OF CELL DIVISION IN *STREPTOMYCES COELICOLOR*

***N. Grantcharova**[1], K. Flärdh[2];*
*[1]Uppsala University, Uppsala, SWEDEN, [2]Lund University, Lund, SWEDEN*

The soil bacterium *Streptomyces coelicolor* A3(2) is a hyphal organism with a complex developmental cycle. *S. coelicolor* A3 (2) undergoes at least two kinds of cell division. Infrequent cross-wall formation takes place in vegetative hyphae and synchronized multiple septation divides sporogenic aerial hyphae into unigenomic spores. Both processes require the FtsZ protein, but are differentially regulated in time and space, and the cell division machinery is developmentally modulated in the different cell-types of *S. coelicolor*. In aerial hyphae, expression of *ftsZ* is strongly upregulated when sporulation is initiated, and this is required for assembly of FtsZ into an array of regularly spaced cytokinetic rings, which eventually orchestrate the formation of the sporulation septa. We used an FtsZ-EGFP translational fusion to visualize the Z-ring assembly *in vivo*. This revealed that synchronized sporulation septation involved formation of helical FtsZ-intermediates and apparently dynamic remodelling of these polymers into Z rings, before initiation of septation. These observations also showed that FtsZ initially polymerised all along the hyphal cell, and no specific nucleation sites (e.g. predetermined sites for Z-ring formation) or zones of inhibition (such as the inhibition of FtsZ assembly by the Min system or the nucleoid veto in other bacteria) were observed. To analyse the Z-ring assembly during sporulation in more detail, we have set up a genetic screen for isolating FtsZ mutants that are specifically defective in sporulation. This exploits the unique properties of *S. coelicolor* as a model for bacterial cytokinesis, such as the dispensability of cell division for growth and survival, the availability of a viable *ftsZ*-null mutant, and the use of the spore pigment as a visual indicator of successful sporulation septation. We have previously reported one such mutant. This allele, *ftsZ17*(Spo), was expressed normally, and the strain carrying it was unable to sporulate and to assemble FtsZ correctly during sporulation, while it was capable of making vegetative septa. We have now isolated several more non-

sporulating FtsZ mutants that confer sporulation defects of variable magnitudes. They are characterized in terms of sporulation phenotypes, effects on FtsZ expression levels, assembly of Z-rings, and effects on DNA segregation, and the positions of mutations are evaluated using a homology model of the *S. coelicolor* FtsZ 3D structure.

# B12

## INFLUENCE OF CARBON SOURCE ON NITROGEN UTILISATION IN *STREPTOMYCES CAPREOLUS*

***M. L. Lea***[1]*, G. Hobbs*[1]*, L. Wigley*[2]*;*
*[1]Liverpool John Moores University, Liverpool, UNITED KINGDOM, [2]Eli Lilly & Company Limited, Liverpool, UNITED KINGDOM*

Tuberculosis has re-emerged as one of the leading causes of death in developing countries worldwide. An estimated 8.89 million new cases emerge each year of which 52,000 cases will result in death, this corresponds to a staggering 7,000 deaths every day.
Capreomycin, an aminoglycoside antibiotic first described in 1960 is primarily effective against the Mycobacteria, but more importantly has become a vital therapeutic agent for the treatment of Multi-Drug Resistant Tuberculosis (MDR-TB), a strain of *Mycobacterium tuberculosis* that does not respond to standard antitubercular drugs such as streptomycin and rifampicin. The bactericidal action of capreomycin is unclear, but is believed to bind to the 30S bacterial ribosome, inhibiting protein synthesis as with other aminoglycosides. Capreomycin comprises four species, capreomycin IA and IB the clinical agents which contain serine and β-lysine and IIA and IIB, minor products of secondary metabolism containing alanine. All components contain α,β-diaminopropionic acid and α(2-iminohexahydro-4-pyrimidyl) glycine, the latter being unique to the capreomycins.
In conjunction with Eli Lilly & Company Limited the main producer of capreomycin, research is being undertaken to gain a better understanding of capreomycin synthesis in sub-merged fermentation with particular interest in the effects of media composition, namely carbon and nitrogen on physiological and biochemical processes.
Glucose is a major carbon source for many antibiotic fermentations and the carbon derived from glucose metabolism in this fermentation is believed to form the structural backbone of capreomycin. This recent study has shown that glucose uptake is significantly reduced in the presence of certain amino acids, suggesting a prominent link between carbon and nitrogen metabolism. This work is significant to ongoing media development and would suggest a cause of antibiotic yield variability currently observed by the employment of complex peptones as nitrogen sources.
Work will also be carried out to investigate sugar transport systems using uptake inhibitors, in addition to preferential sugar utilisation and $K_m$ determination. The effects of $K^+$ and $Na^+$ on amino acid transport in *S. capreolus* will also be examined alongside the effects of pH on these systems.

# B13

## *MYXOCOCCUS XANTHUS* DEVELOPMENT AND VEGETATIVE GROWTH UNDER COPPER STRESS

*A. Moraleda-Muñoz, N. Gómez-Santos, M. Sánchez-Sutil, R. García-Hernández, J. Munoz-Dorado,* ***J. Pérez****;*
*Universidad de Granada, Granada, SPAIN*

*M. xanthus* glides through the soil either as single bacteria, or more typically, as groups of bacteria feeding on whichever bacterial prey they can lure into their vicinity. However, under conditions of nutrient depletion, it undergoes a developmental program, unique among the prokaryotes, in which cells come together in a coordinated way to produce multicellular fruiting bodies within which they sporulate converting the vegetative cells into myxospores. This aggregation and differentiation of individual bacteria requires a complex interaction of signals controlling gene expression in time and space. *M. xanthus* is common in soils rich in organic material and rotting-wood. In those niches this bacterium has to cope frequently with high levels of aromatic compounds resulting from lignin degradation and toxic concentrations of metal ions from industrial pollution. Copper is an essential trace element, but an excess is toxic especially for lower organisms because it is able to catalyze cytotoxic reactions. We have recently found that copper induces the accumulation of carotenoids in dark-grown cultures of *M. xanthus* activating the transcription of the structural genes for carotenoid synthesis (Moraleda-Muñoz *et al.* Molecular Microbiology, in press). This study has revealed that, in addition to carotenogenesis, copper induces other unknown cellular mechanisms that confer tolerance to this metal. We are presently studying three different operons (*curA*, *curB* and *curC*) structurally located in regions nearby of the chromosome. These operons seem to code for a multi-layered process not only regulating uptake and efflux but also periplasmic copper sequestration. Operons *curA*, *curB* and *curC* contain genes coding for three different periplasmic multicopper oxidases (MCOs) that we have named LcsA, LcsB and LcsC, respectively. Operons *curA* and *curB* are induced by copper while *curC* is not. The amount of copper required to obtain the same levels of expression of *curA* and *curB* operons is 10-fold lower during development than during vegetative growth. Characterization of in-frame deletion mutants in the MCO genes and regulation of the *curA* operon by a $\sigma^{54}$-dependent two-component regulatory system (*cusS*/*cusR*) will be presented at the conference.

# B14

## GENETIC AND PHYSICAL INTERACTION OF *BACILLUS SUBTILIS* TOPOISOMERASES I AND IV WITH SMC PROTEIN

***S. Tadesse Telila***[1]*, J. Mascarenhas*[2]*, B. Kösters*[2]*, A. Hasilik*[2]*, P. L. Graumann*[1]*;*
*[1]Albert-Ludwig Universität, Freiburg, GERMANY, [2]Philipps-Universität Marburg, Marburg, GERMANY*

Visualization of topoisomerases I and IV in live *Bacillus subtilis* cells showed that Topo I and IV differentially localize on the nucleoids, but are absent from cytosolic spaces surrounding the nucleoids, suggesting that these topoisomerases interact with many regions of the chromosome. Topo I formed discrete centres on the nucleoids in a large fraction of cells, which frequently co-localized with SMC protein. Localization of Topo I was aberrant in *smc* mutant cells, and FRET experiments showed a direct interaction of SMC and Topo I. Lowering of the levels of Topo IV led to chromosome decondensation, while its overproduction induced hyper-compaction of chromosomes, showing that Topo IV has an influence on condensation of the whole chromosome, similar to SMC. Increased synthesis of Topo IV in *smc* deleted cells partially rescued the growth and condensation defect, but not the segregation defect of the deletion, revealing a genetic interaction between the two systems. 2D gel investigations showed that global protein synthesis is highly aberrant in *smc* deleted cells, and, to a different extent, also in cells lacking ScpA or ScpB, which form the SMC complex together with SMC protein. Overproduction of Topo IV rescued the defect in protein synthesis in *smc* mutant cells, indicating that Topo IV can restore the loss of negative supercoiling in the absence of SMC, but does not fully rescue the segregation defect. The data show that SMC has a dual function, in chromosome supercoiling and in active segregation

# B15

## ZINC-DEPENDENT DIFFERENTIAL EXPRESSION OF THE GENES, *RPSN* AND *YHZA*, CODING FOR TWO TYPES OF S14 PROTEIN IN *BACILLUS SUBTILIS*

***Y. Natori****, G. Akanuma, H. Nanamiya, F. Kawamura;*
*Rikkyou university, Tokyo, JAPAN*

Among 57 ribosomal proteins of *Bacillus subtilis*, 5 proteins (L31, L32, L33, L36 and S14) contain a Cys-xx-Cys motif, which is thought to be a zinc binding motif. Moreover, two genes, *ytiA* (YtiA), and *yhzA* (S14-2) are assigned to encode the paralogous gene products of L31 (RpmE) and S14 (RpsN) respectively, but their products do not contain a Cys-xx-Cys motif. We have found that alternation of two types of L31 protein is regulated by zinc. We next examined whether or not the expression of the two types of S14 protein is regulated by a mechanism similar to that of the two types of L31 protein. The *rpsN* gene is found in the S10-*spc* operon which contains 21 ribosomal protein genes in *B. subtilis*. Since the S10-*spc* operon is highly conserved among prokaryotes, it was assumed that the *rpsN* gene was essential for growth of *B. subtilis*. To test this, an intact *rpsN* gene, whose transcription was controlled by an IPTG inducible P*spac* promoter, was inserted at the *aprE* site of the chromosome. Next, the original *rpsN* gene was deleted by replacing the *rpsN* gene with a *cat* gene in the strain. Using this strain, we found that cell growth was dependent on the addition of IPTG. Moreover, depletion of RpsN caused a severe effect on 70S formation. These results indicated that RpsN was essential for cell viability. To examine whether YhzA complements the growth defect of an *rpsN* null mutation, we tried to introduce the *rpsN* deletion mutation into the strain carrying the P*spac*-controlling *yhzA* gene inserted at the *aprE*. As the results, transformants were successfully obtained when IPTG was added to the medium. However, cells grew slowly compared with those cells with the P*spac*-controlling *rpsN* gene, even though the same concentration of IPTG was used. Moreover, although the RpsN protein was found in the crude ribosome fraction even after the expression of *yhzA* was fully increased, the transcription of *yhzA* was negatively regulated by Zur as that of *ytiA*. These results suggest that, unlike RpmE and YtiA, RpsN plays an essential role for the ribosome function as the major S14 protein, and the YhzA protein functions as the S14 protein only when the RpsN protein is not present in the cell.

# B16

## FUNCTIONAL ANALYSIS OF PBP2B IN *BACILLUS SUBTILIS*

***M. Xu****, J. Errington, R. A. Daniel;*
*University of Oxford, Oxford, UNITED KINGDOM*

*Bacillus subtilis* is the most widely studied Gram-positive bacterium and has served as a model prokaryotic system for the investigation of cell division. In *B. subtilis*, at least eight gene products, FtsZ, EzrA, ZapA, FtsA, FtsL, DivIC, DivIB, and PBP2b have been identified as part of the division machinery in vegetative and sporulating cells. PBP2b (encoded by *pbpB*) of *B. subtilis* belongs to the class B high molecular weight family of PBPs, and is required for cell septation. PBP2b is a bitopic protein composed of 713 amino acid residues, and can be divided into two domains, a C-terminal transpeptidase domain and an N-terminal domain with undefined function. Immunofluorescence microscopy (IFM) analysis of the assembly of the division complex in *B. subtilis* showed that FtsL, DivIB, DivIC and PBP2b target to the division site after FtsZ ring is formed. Mutations in *ftsL*, *divIB*, and *divIC* prevented PBP2b from localizing to the division site; similarly, depletion of *ftsL* had little or no effect on FtsZ ring formation, but the assembly of other division protein, DivIB, DivIC and PBP2b, at the division site was prevented. This

potentially indicated that the assembly may involve close interactions of division proteins DivIC, DivIB, FtsL and PBP2b, which raised the possibility that the N-terminal domain of PBP2b could act in these interactions. To investigate this possibility, twin-alanine scanning mutagenesis of PBP2b of N-terminal domain has been carried out to search for important sites in this domain. Some of the mutants were found to have interesting phenotypes and were investigated in more detail. The *pbpB* mutations were combined with characterised conditional mutations in other division genes to understand the potential interaction between PBP2b and other cell division proteins. The results showed that twelve mutants were incompatible with a *divIB* null mutation, and most of these mutants were also incompatible with the *divIC*$^{355}$ TS mutation. *ftsL* overexpression was found to suppress some mutant phenotypes. It was also observed that two of the phenotypeless *pbpB* mutations can exacerbate the *divIB* and *divIC* mutant phenotypes. The active *pbpB* mutations define four regions in the N-terminal domain of PBP2b that may participate in its interaction with DivIB, DivIC, and FtsL.

# B17

## HEAVY METAL CONTROL OF MORPHOLOGICAL DEVELOPMENT REVEALED BY THE EXTRACELLULAR PROTEOME OF STREPTOMYCES LIVIDANS

***E. Vijgenboom**, S. Bialek, B. J. Keijser, G. W. Canters, E. C. de Waal, M. C. Machczynski;*
*Leiden University, Leiden, THE NETHERLANDS*

The switch from vegetative to aerial growth induces significant changes in the extracellular proteome of Streptomyces. These changes are good markers for the identification of extracellular factors that are important for development. We designed growth conditions that allow complete control over the developmental switch from vegetative to aerial growth in Streptomyces lividans by altering the availability of Cu-ions in the medium. Comparison of the extracellular proteomes of surface grown cultures fixed in vegetative growth (no Cu available) with those producing aerial hyphae (≥ 0.2 µM Cu2+), revealed that upon arresting development in vegetative growth, several heavy metal homeostasis related proteins are expressed among which Sco1 and proteins of the iron-siderophore uptake system. The copper chaperone Sco1 is required for the maturation of the respiratory terminal oxidase cytochrome c oxidase, COX. This oxidase is responsible for the transfer of electrons to oxygen and the concomitant generation of ATP under oxygen rich conditions. A S. lividans knock-out mutant of sco1 is devoid of COX activity and has a bald phenotype in contrast to the wild type strain that has high COX activity and full development. However, a cox knock-out mutant is still capable of development albeit significantly slower that the wild type strain. The latter suggests that Sco1 must have other activities in addition to the maturation of COX. The effect of the cox mutation on aerial hyphae production shows that respiration and development are tightly linked. The appearance of siderophore uptake related proteins signals a severe iron shortage in the mycelium. This iron shortage could be a development related feature or the result of the Cu limitation. The latter is likely since a direct relation between iron- and copper homeostasis has been demonstrated in several organisms. In S. cerevisiae a copper protein is an essential subunit of the iron (II) uptake system. However, a homologue has not been found on the S. coel genome. A gene knock-out approach is in progress to determine if one of the membrane bound or extracellular Cu-proteins could be involved in the iron-copper link in Streptomyces. Maintenance of the proper iron concentration in the mycelium is important for development because Streptomyces growth on iron limited media is blocked in the vegetative growth phase. Data will be presented on the changes in the extracellular proteome of S. lividans in relation to the availability of the redox metals iron and copper.

# B18

## TRANSCRIPTIONAL FACTORS DURING SPORULATION IN BACILLUS SUBTILIS

***R. Kuwana**, H. Takamatsu, K. Watabe;*
*Setsunan University, Hirakata, Osaka, JAPAN*

Endospore formation in Bacillus subtilis involves a series of temporally and spatially ordered changes in cell morphology and gene expression. During sporulation in B. subtilis, differential gene expression in the prespore and the mother cell compartments is governed by four sporulation-specific sigma factors. Two sigma factors, SigF and SigG, are active specifically in the prespore, while SigE and SigK control the transcription in the mother cell. The transcriptional regulators SpoIIID and GerE is transcribed by SigE or SigK respectively, and activates or represses the transcription of a group of SigE and/or SigK dependent genes in the mother cell compartment. The sporulation-specific YlbO and SpoVIF are small soluble proteins and essential for the development of heat and lysozyme resistant spores. Analysis of protein composition of the ylbO and spoVIF negative mutant spores indicated that coat proteins, which are positively regulated by GerE, were not present in them. Northern blotting showed that YlbO and SpoVIF regulated the expression of the genes at transcriptional level. We suggest that more two proteins YlbO and SpoVIF regulate the expression of spore coat proteins during sporulation in Bacillus subtilis.

# B19

## A NOVEL CYTOSKELETAL PROTEIN OF *STREPTOMYCES COELICOLOR* A3(2)

*A. Thibessard[1], A. Tzanis[1], S. Cantlay[1], K. Findlay[2],* ***G. Kelemen[1];*** *[1]University of East Anglia, Norwich, UNITED KINGDOM, [2]John Innes Centre, Norwich, UNITED KINGDOM*

One of the most fascinating aspects of *Streptomyces* biology is its complex cell cycle, which makes it a model organism for bacterial development. Unlike most bacteria that divide by binary fission, in *Streptomyces coelicolor* long, multigenomic filaments are formed with occasional septa and regular branching. Cell division is completed only during sporulation when 50-100 sporulation septa are laid down synchronously in the aerial hyphae generating unigenomic spore compartments. Cytoskeletal proteins have been shown to be involved in cell division and in controlling cell shape in several bacteria. The spatial characteristics of the long hyphal compartments of *S.coelicolor* raised the question whether cytoskeletal proteins were involved in molecular transport during polarized hyphal growth.
We have identified a putative cytoskeletal protein, Scy, in *Streptomyces coelicolor*. The morphology of the *scy* mutant is severely affected: showing uneven hyphal diameter, shortened hyphal length, unusual branching pattern and altered chromosome distribution during sporulation. Transcription of *scy* is detected at all stages of differentiation. Monitoring the localization of Scy-EGFP translational fusion during development together with immunological detection of Scy will also be shown.

# B20

## COMPARATIVE STUDY ON TWO PUTATIVE *LUXR* GENES IN THERMUS ISOLATES

***L. Lin****, G. Lin;*
*Tzu-Chi University, Hualien, TAIWAN REPUBLIC OF CHINA*

A thermophile is an organism which thrives at high temperatures. Thermophiles have been found in various geothermal heated regions of the earth. Extensive studies focus on the application of thermo-stable enzymes in the past years. Our research interest is the mechanism of biofilm formation in thermophilic isolates. Nature biofilm communities are characterized by spatial and temporal heterogeneity in biofilm architecture, populations and metabolic capabilities. Quorum sensing and the production of autoinducers allow bacteria to coordinate certain metabolic activities. It may play an important role in the development and succession of biofilm communities. Our previous studies discover that, by using conditional medium to culture non-biofilm thermophilic isolates can lead to biofilm formation. Further purification and bio-activity assay prove the exist of one molecule involving in the growth of biofilm. LC-MS profile gives some evidences to postulate the autoinducer role of this molecule. Searching the completed sequence of *Thermus thermuphilus* HB27, we find out two putative LuxR family proteins. LuxR has been considered to regulate the targeted genes under controlling of quorum sensing signals. This plan aims to disrupt the putative *lux*R genes in *Thermus thermuphilus* HB27 and *Thermus aquaitu*s. Comparative analysis will help to understand the effect of these genes on the growth of biofilm.

# B21

## CHROMOSOME ORGANISATION IN *STREPTOMYCES COELICOLOR* A3(2)

***S. Cantlay[1]****, A. Thibessard[1], K. Findlay[2], G. Kelemen[1];*
*[1]University of East Anglia, Norwich, UNITED KINGDOM, [2]John Innes Centre, Norwich, UNITED KINGDOM*

*Streptomyces coelicolor* has a complex developmental life cycle, from germination and vegetative mycelial growth, through to the development of aerial hyphae and spore formation. Here we focus on how the organism organises its genetic material during this development and, in particular, during the transition from multigenomic hyphae to unigenomic spores. A single chromosome of *S.coelicolor* is linear with centrally located origin of replication and covalently bound proteins at its ends. However, it has been shown that the ends of the chromosomes are held together, effectively forming a circular chromosome. The state of the nucleoid changes during development as chromosomes within mature spores are tightly condensed compared to that within growing hyphae. Although extensive studies have been carried out on *Streptomyces* development focusing on the formation of aerial hyphae (*bld* genes) and the initiation and completion of sporulation (*whi* genes), no previous studies have addressed some basic questions concerning hyphal morphogenesis such as how chromosomes are positioned within hyphae that are continuously extending at their tip and whether chromosome packaging plays regulatory role during the different stages of development?
To address some of these questions we generated a series of mutants using PCR targeted disruptions of genes encoding chromosome associated proteins like Smc (Structural Maintenance of Chromosomes), ScpA and B (proteins potentially associated with Smc) and ParH, a protein homologous to ParA. Phenotypic analysis of the mutant strains revealed distinct morphological characteristics of the *smc* and the *parH* mutants and transcription of both *smc* and *parH* genes were shown to be developmentally regulated. Localisation of Smc and ParH during colony development will be shown.

# B22

## COMPARISON OF THE ALTERNATIVE DESIGNS FOR THE PARTNER SWITCHING SIGNALING NETWORKS CONTROLLING THE SIGMAB AND SIGMAF FACTORS IN *BACILLUS SUBTILIS*

***O. A. Igoshin***, *C. W. Price, M. A. Savageau;*
*University of California, Davis, CA*

Regulatory networks controlling bacterial gene expression often evolve from a common origin and, therefore, involve homologous proteins and share similar network motifs. For example, in *Bacillus subtilis* the activities of both the stress response factor $\sigma^B$ and the first sporulation-specific factor $\sigma^F$ are controlled by similar partner switching mechanisms. However, clear differences in network organization are apparent: the anti-sigma-factor in the $\sigma^F$ network is known to form a long-lived, "closed" state with its antagonist and ADP; and the genes for $\sigma^B$ and its network partners lie in a $\sigma^B$-controlled operon, resulting in both positive and negative feedback loops. Here we compare these alternative designs for partner-switching signaling networks. We constructed mathematical models of both networks and performed mathematically controlled comparisons. The results of this analysis suggest how differences in network organization correlate with different physiological demands. Existence of the "closed" state in the $\sigma^F$ network is predicted to produce a largely irreversible hysteretic switch that is appropriate for the commitment to differentiation. On the other hand, the feedback loops in the $\sigma^B$ network are predicted to produce continuously-graded, reversible behavior over a larger range of regulation and with a faster response that is appropriate for a system that must accommodate highly variable stresses. These results demonstrate how alternations in network design can result in qualitatively and quantitatively different system properties, and they make clear predictions that can be experimentally tested.

# B23

## PROTEIN-PROTEIN INTERACTIONS INVOLVING THE CAULOBACTER CRESCENTUS POLAR DEVELOPMENT PROTEIN PODJ

***D. Klein***, *Y. Brun;*
*Indiana University, Blomington, IN*

*Caulobacter crescentus* exhibits an inherently asymmetric life cycle. A newly formed pole, produced at the site of cell division, will first develop into a swarmer pole by assembling pili and a flagellum. After a set period of time this swarmer pole will shed the flagellum, retract the pili, and synthesize a narrow extension of the cell body, called a stalk, tipped by an adhesive structure called the holdfast. Production of the stalk and holdfast marks the end of the polar differentiation program. A number of genes have been identified in pathways controlling this process. One such gene, *pleC*, codes for a histidine protein kinase. Another such gene, *podJ*, does not share homology to any known proteins, but does have several regions with homology to known protein domains typically involved in protein-protein interactions. A *pleC* mutant does not make a holdfast or a stalk, has a paralyzed flagellum, and does not synthesize pili. A *podJ* mutant also fails to make a holdfast or pili, but does make a stalk and an active flagellum, although their ability to swim in semi-solid agar is impaired relative to wild-type. Both PodJ and PleC exhibit distinct polar localization phenotypes - both proteins localize to the future swarmer pole in stalked cells. Furthermore, PleC localilzation is dependent on the presence of PodJ. The N-terminal, cytoplasmic region of PodJ has shown to be sufficient for holdfast synthesis and for localization of itself and of PleC to the swarmer pole. The similarity of the expression and localization profiles of PleC and PodJ, and the presence of predicted protein-protein interaction domains in PodJ suggest that PodJ may be responsible for localizing PleC via a direct interaction. To determine if PodJ and PleC interact with each other or with themselves, yeast two-hybrid vectors expressing the full-length proteins (PodJ-full and PleC-full) as well as the cytoplasmic ( -cyt) and periplasmic ( -peri) regions of the proteins as fusions with the activation and DNA-binding domains of the GAL4 yeast transcriptional activator have been constructed. Interaction has been successfully shown between PodJ-full and PodJ-full, between PodJ-cyt and PodJ-cyt, and between PodJ-full and PodJ-cyt. No interaction has been detected in any combination with PodJ-peri or with any form of PleC. However, this may be a result of poor or faulty protein expression in yeast rather than an indication of no true interaction.

# B24

## EXAMINATION OF THE RELATIONSHIP BETWEEN GLIDING MOTILITY AND THE CYTOSKELETON-LIKE MATERIAL IN THE *MYCOPLASMA PNEUMONIAE* PHYLOGENETIC GROUP

***J. M. Hatchel***, *D. A. Jurkovic, R. Swanson Balish, M. F. Balish;*
*Miami University, Oxford, OH*

Mycoplasmas, many of which are pathogens, are members of the bacterial class *Mollicutes*. These organisms, which evolved from gram-positive bacteria, have reduced genomes, lack cell walls, and are the smallest organisms capable of growth in pure culture despite fastidiousness in growth requirements. Despite this simplicity, at least some mycoplasmas are ultrastructurally complex, with a polar extension called the attachment organelle. Through the attachment organelle, the human pathogen *Mycoplasma pneumoniae* as well as some other

mycoplasmas attaches to host cells; additionally, the attachment organelle is the leading end of the cells during gliding motility, a process of likely pathogenic significance but unknown mechanism. The *M. pneumoniae* attachment organelle is characterized by the presence of a Triton X-100-insoluble electron dense core which, together with other fibers, constitutes a cytoskeleton-like structure which is required for normal cellular morphology, attachment to host cells, and virulence. The presence of the attachment organelle at the leading end of the cell during gliding motility has led to the suggestion that, analogous to eukaryotic cells, the cytoskeleton-like structure within the *M. pneumoniae* attachment organelle is associated mechanistically with gliding. The closely related avian respiratory pathogen *Mycoplasma gallisepticum* also features an electron-dense core, but *Mycoplasma penetrans*, isolated from the urogenital tract of AIDS patients, is reported to lack such a feature; in neither species are the ultrastructure of the cytoskeleton or the gliding motility characteristics well-described. In order to characterize the cytoskeletons of *M. gallisepticum* and *M. penetrans*, both of which are members of the *M. pneumoniae* phylogenetic group, with reference to *M. pneumoniae,* we have begun to examine the Triton X-100-insoluble material and gliding motility characteristics of these organisms using scanning electron microscopy, time-lapse microcinematography of phase-contrast images, and biochemical fractionation. Our results suggest that the electron-dense core of *M. gallisepticum* is similar in structure to that of *M. pneumoniae*, whereas we do not observe any kind of cytoskeleton-like structure in *M. penetrans* despite rapid motility. Assuming that these closely related organisms use the same mechanism for motility, these data call into question any direct relationship between the cytoskeleton of *M. pneumoniae* and the mechanism underlying gliding motility. Alternatively, *M. penetrans* might use an entirely different mechanism for gliding despite a close phylogenetic relationship with *M. pneumoniae* and *M. gallisepticum*.

# B25

## COMPLEXITIES OF GENE REGULATION IN RESPONSE TO C-SIGNALING DURING *MYXOCOCCUS XANTHUS* DEVELOPMENT

*P. Viswanathan, D. R. Yoder, K. Viswanathan,* ***L. Kroos****;*
*Michigan State University, East Lansing, MI*

Studies of C-signaling during *M. xanthus* development are establishing a new paradigm for how bacterial cells can interact. C-signaling involves CsgA, a protein produced in the developing cells that becomes associated with the cell surface. It has been proposed that a proteolytic fragment of CsgA serves as a signal upon end-to-end contact between cells. C-signaling induces several responses in recipient cells. It regulates cell movements, allowing macroscopic patterns called ripples and aggregates to form, and it regulates developmental gene expression and sporulation. Intriguingly, the different responses to C-signaling require different levels of CsgA, and the level of CsgA rises during development. Therefore, C-signaling might ensure that certain genes are activated earlier, during the aggregation stage, while others are activated later, triggering sporulation. Some genes, like *4403*, depend absolutely on C-signaling for expression, others, like *4400*, depend only in part. We have identified *cis*-acting DNA elements in the promoter regions of C-signal-dependent genes. Surprisingly, the identical element functions differently in the *4403* and *4400* promoter regions. This element, called the C box, is also found in the promoter region of at least one developmental gene (*fruA*) whose expression does not depend on C-signaling, and mutational analysis suggests it functions differently there than in either of the C-signal-dependent promoter regions. FruA is a response regulator that mediates cellular responses to C-signaling. We now report that the FruA DNA-binding domain binds to a region that includes the C box in the *4403* promoter region, whereas it binds slightly farther upstream in the *4400* promoter region near -80 bp, and much farther upstream (near -320 bp) in the *dev* promoter region (whose expression depends partially on C-signaling). Interestingly, the *dev* promoter, as well as the *4406* promoter (whose expression depends absolutely on C-signaling), require DNA downstream of the transcriptional start site for expression. The *4406* promoter is regulated negatively *via* an element located between -500 and -100 bp, which mediates C-signal dependence. Another unexpected complexity revealed by our studies is that expression of certain genes depends strongly on their position in the chromosome.

# B26

## DECIPHERING THE FUNCTION AND TOPOLOGY OF TWO-COMPONENT SIGNALING NETWORKS IN *CAULOBACTER CRESCENTUS*

***S. Crosson****;*
*Stanford University School of Medicine, Stanford, CA*

Cells have the remarkable ability to modulate their physiology in response to changes in their environment. Although *Caulobacter crescentus* encodes at least 64 sensor histidine kinases, the signals that activate these kinases are not generally known. To date, I have identified two cofactor-binding histidine kinases in *Caulobacter* that act as environmental sensors. The first, FixL, measures dissolved oxygen concentration via a heme cofactor and acts to modulate the expression of genes encoding multiple respiratory terminal oxidases and metabolic enzymes. In addition, FixL regulates the transition of *Caulobacter* from free-living cells into a multicellular biofilm. The second kinase, HK5, binds a flavin cofactor, serves as a sensor of blue light, and appears to regulate the transcription of DNA repair enzymes. In collaboration with Michael Marletta at Berkeley, I am also characterizing another hemoprotein sensor, cHNOX, that specifically binds nitric oxide. This gas sensor is co-transcribed with the hybrid histidine kinase/response regulator, CC2993, which regulates the biosynthesis of methionine and the repair of oxidatively-

damaged methionine. This suggests that cHNOX may modulate methionine biosynthesis and repair in response to reactive nitrogen stress. These studies promise to provide insight into how *Caulobacter* receives and integrates signals from its environment to generate the appropriate physiological, metabolic, or developmental response.

# B27

## THE ROLE OF THE STRINGENT RESPONSE IN THE BACTERIUM SORANGIUM CELLULOSUM

***T. Knauber**, S. Doß, A. Treuner-Lange;*
*University of Giessen, Giessen, GERMANY*

The myxobacteria are a group of strictly aerobic, mesophile gram-negative soil bacteria. Which phylogenetically belong to the δ group of proteo bacteria. Myxobacteria are of interest because of their complex life cycle and their secondary metabolite production. Under starvation conditions the myxobacteria aggregate, pile up and finally form multicellular structures called fruiting bodies. The myxobacteria also produce bioactive secondary metabolites, which often show novel chemical structures of interesting mechanisms of action. Most of these compounds appear to be developed to inhibit eukaryotic competitors in the soil habitat. Within the myxobacteria Sorangium species are the most important secondary metabolite producer. Therefore the genome of S. cellulosum is currently analyzed. In Streptomyces, which also undergo complex processes of morphological and physiological differentiation, it could be shown that both processes are initiated by the stringent response. We wanted to know if the same regulatory connection exists in S. cellulosum and started to analyse the stringent response in S. cellulosum. The relA-gene is known to be involved in the stringent response dependent pp(p)Gpp synthesis and hydrolysis. Interestingly the same gene is involved in the onset of differentiation in Myxococcus xanthus, the best studied myxobacterium so far.We constructed a rel insertion mutant and are currently investigating the consequences of that insertion. To analyse the ppGpp production in our rel-mutant we did TLC analyses. These analyses revealed that the production of ppGpp is severly reduced in the rel-mutant compared to the wildtype. To answer the question if the production of the secondary metabolites is under stringent control qualitative as well as quantitative analyses were performed. Indeed, the mutant is impaired in maximal production of these secondary metabolites. To see if the reduction of chivosazole production is based on the fact that the corresponding genes are not expressed in the mutant we did Realtime RT-PCR. These experiments indicated that the reduced production in the mutant is the consequence of a transcriptional defect. That observation suggest that specific transcriptional activators, which are required for full expression of the corresponding operon are activated under stringent control and therefore we started 2-D-gel as well as DNA binding proteins analysis to identify those putative transcription factors. Also a differentiation defect could be observed in the rel-mutant under starvation conditions, suggesting that the morphological as well as physiological differentation processes in S. cellulosum are initiated by the stringent response like it is known from Streptomyces spec.

# B28

## THE INTERACTION BETWEEN MORPHOGENETIC PROTEINS SPOVID AND SAFA DURING SPORE COAT ASSEMBLY IN *BACILLUS SUBTILIS*

***T. Costa**[1], A. J. Ozin[2], C. P. Moran, Jr.[2], A. O. Henriques[1];*
*[1]Instituto de Tecnologia Química e Biológica, Oeiras, PORTUGAL, [2]Emory University School of Medicine, Atlanta, GA*

During formation of the *Bacillus subtilis* endospore coat a class of morphogenetic proteins acts by guiding the assembly of a large number of structural components to the surface of the developing spore. SpoVID for example, plays a key role in coat morphogenesis: in *spoVID* mutants the coat structural components form swirls of partially structured material throughout the mother cell cytoplasm, and the resulting spores have an exposed cortex peptidoglycan (PG) and are highly susceptible to lysozyme. SpoVID is required for the correct localization of another morphogenetic protein, SafA, around the developing spore. *safA* spores have deficient coats and are also susceptible to lysozyme. A cell wall binding (CWB) motif (a LysM domain) is present at the C- or N-terminal regions of SpoVID and SafA, respectively, and both proteins localize near the outer membrane of the developing forespore, close to the underlying cortex PG. *In vitro* and *in vivo* assays showed that SpoVID and SafA directly interact. We found that the CWB motif in both SpoVID and SafA is not necessary for their interaction. We also found that residues 51 to 63 of SafA, just downstream of the CWB motif, are involved in the interaction with SpoVID. Deletion of the 51-63 region, of residues 51-57 or 58-63, impairs the SpoVID-SafA interaction in vitro, and results in spores with an aberrant coat that are susceptible to lysozyme. A quadrupule alanine substitution within the 51-63 amino acid region also impairs the interaction of SpoVID with SafA *in vitro*, and causes the assembly of an altered coat *in vivo*. However, the resulting spores are resistant to lysozyme, suggesting that the alanine substitutions within the 51-63 aa region of SafA permit some degree of interaction with SpoVID *in vivo*. All the mutant forms of SafA accumulate to normal levels in sporulating cells, and undergo assembly into the spore coat. We conclude that the interaction of SpoVID with the region comprising residues 51-63 of SafA is essential for proper coat assembly.

## B29

### IDENTIFICATION AND CHARACTERIZATION OF A C-DI-GMP SPECIFIC PHOSPHODIESTERASE AND ITS ALLOSTERIC CONTROL BY GTP

***M. Christen**, B. Christen, A. Schauerte, M. Folcher, U. Jenal;*
*University of Basel, Basel, SWITZERLAND*

Cyclic diguanylic acid (c-di-GMP) is a global secondary messenger controlling motility and adhesion in bacterial cells. Synthesis and degradation of c-di-GMP is catalyzed by diguanylate cyclases (DGC) and c-di-GMP-specific phosphodiesterases (PDE), respectively. While the DGC activity has recently been assigned to the widespread DUF1 or GGDEF domain, the enzymatic activity responsible for c-di-GMP cleavage in bacterial cells has been associated with proteins containing a DUF2 or EAL domain. Here we show biochemically that CC3396, a GGDEF-EAL composite protein from *C. crescentus* is a soluble PDE. The PDE activity, which rapidly converts c-di-GMP into the linear dinucleotide pGpG, is confined to the C-terminal EAL domain of CC3396, depends on the presence of $Mg^{2+}$ ions and is strongly inhibited by $Ca^{2+}$ ions. Remarkably, the associated GGDEF domain lacks detectable DGC activity. Instead, GGDEF is able to bind GTP and in response activates the PDE activity in the neighboring EAL domain. PDE activation is specific for GTP (Kd 4 μM) and operates by lowering the Km for c-di-GMP of the EAL domain to a physiologically significant level (420nM). Mutational analysis suggested that the substrate-binding site (A-site) of the GGDEF domain is also required for the GTP-dependent regulatory function, arguing that a catalytically inactive DGC domain can still bind GTP and in response activate the neighboring PDE domain. Based on this we propose that the c-di-GMP-specific PDE activity is confined to the EAL domain, that GGDEF domains can either catalyze the formation of c-di-GMP or can serve as regulatory domains and that c-di-GMP-specific phosphodiesterase activity is coupled to the cellular GTP level in bacteria.

## B30

### A DOMINO MODEL FOR THE SWITCHING MECHANISM OF THE FLAGELLAR MOTOR OF *CAULOBACTER CRESCENTUS*

***G. Li**[1], Q. Wen[1], G. Huber[2], J. X. Tang[1];*
*[1]Brown University, Providence, RI, [2]University of Connecticut Medical Center, Farmington, CT*

Switching of the rotation direction of the flagellar motor plays a crucial role in the chemotaxis of flagellated bacteria, yet it remains poorly understood. Our current knowledge of flagellar motor mechanics stems from studies of *E. coli*. Here, we study the switch of the flagellar motor of *Caulobacter crescentus*, a Gram-negative bacterium whose flagellar motor is similar to that of *E. coli*. A peak is observed in the distribution of the time intervals of clockwise (CW) or counterclockwise (CCW) rotation. We propose a domino-toppling model to explain the switching mechanism. In this model, the noise induces FliM to switch between CCW and CW conformations. The switch of an individual FliM causes the switch of the FliM next to it, and, hence, all the other FliMs in the motor's C-ring, like the collapse of a ring of dominoes. The binding of CheY-P changes the switching ability of a FliM. The simulated interval distributions based on this model agree with those of the Caulobacter flagellar motor. This model can also be applied to explain the observed dependence of CW bias and switching frequency on the CheY-P concentration and the temperature dependence of motor switching as measured for *E. coli*. Different from other models, this model predicts that the dynamic binding of CheY-P to FliM is key to the signal amplification of the motor.

## B31

### CHARACTERIZATION OF *SINORHIZOBIUM MELILOTI* TRIOSE PHOSPHATE ISOMERASE GENES: EVIDENCE FOR A NOVEL PLANT PHENOTYPE

***N. J. Poysti**, I. J. Oresnik;*
*University of Manitoba, Winnipeg, MB, CANADA*

Mutants of *Rhizobium leguminosarum* unable to use rhamnose as a carbon source are less competitive than the wild-type strain for root nodule formation. In order to determine if rhamnose catabolic mutants of *Sinorhizobium meliloti* are similar in nature, a Tn*5* mutagenesis of the *S. meliloti* wild-type Rm1021 was carried out and mutants were screened for the inability to grow on rhamnose as a sole carbon source. One *S. meliloti* mutant was isolated that grew slower than wild-type on rhamnose as a sole carbon source and did not grow on glycerol. The site of the mutation was identified and shown to interrupt the gene *tpiA1*. This gene is predicted to encode one of two annotated triose phosphate isomerases (designated *tpiA1* and *tpiA2*) in the genome of Rm1021. To determine if both *tpi* genes encoded triose phosphate isomerases, mutants of *tpiA2* were generated and both *tpiA1* and *tpiA2* mutants were characterized. The results show that both genes encode triose phosphate isomerase enzymes. *tpiA1* appears to be associated with Embden-Meyerhof-Parnas pathway whereas *tpiA2* is necessary for erythritol catabolism. If a *tpiA1* mutant is grown on glycerol supplemented with 0.4 mM erythritol its inability to grow on glycerol was suppressed due to the induction of *tpiA2* providing *in vivo* evidence that *tpiA2* encodes an inducible triose phosphate activity. A strain containing mutations in both *tpiA1* and *tpiA2* was constructed and it was found that the resultant strain had carbon utilization

phenotypes that were not associated with either *tpiA1* or *tpiA2*. When nodulation competition experiments were carried out with the *tpiA1* mutant, it was found that although these mutants had a delayed growth phenotype on rhamnose, they were as competitive for nodule occupancy as the wild-type. Interestingly alfalfa plants grown under a nitrogen deficient regime and inoculated with *tpiA1* mutants accumulated more dry matter than those inoculated with the wild-type. We hypothesize that this plant phenotype is due to an altered flux of gluconeogenic carbon that occurs within the bacteroid during active nitrogen fixation.

# B32

## PYRUVATE KINASE OF THE MYXOBACTERIUM *STIGMATELLA AURANTIACA* IS ESSENTIAL FOR MULTICELLULAR DEVELOPMENT

*I. Stamm[1], F. Lottspeich[2],* ***W. Plaga[1];***
*[1]ZMBH, University of Heidelberg, Heidelberg, GERMANY, [2]Max Planck Institute of Biochemistry, Martinsried, GERMANY*

Vegetative cells of *Stigmatella* are triggered by starvation to build up a complex multicellular fruiting body consisting of a stem, branches and sporangioles which contain the myxospores. The subroutine of spore formation can be artificially uncoupled from multicellular development by indole and some indole derivatives. Pyruvate kinase and aldehyde dehydrogenase were isolated as putative indole receptors exploiting their capacity to bind indole. The identification of enzymes of the central metabolism as putative targets of the indole effect is paralleled by the reported interaction of the hydrophobic thyroid hormone with pyruvate kinase and aldehyde dehydrogenase in higher organisms.
The functionality of the pyruvate kinase was demonstrated by enzyme assays which also disclosed a stimulation of the pyruvate kinase activity by indole. The gene encoding pyruvate kinase (*pykA*) was cloned and a *pykA* disruption mutant was created which was unable to build up fruiting bodies and was delayed in indole-induced spore formation. When *pykA* was introduced ectopically into the genome of the disruption mutant the complemented strain showed fruiting body formation as the wild type and the delay of indole-induced spore formation was diminished. These results prove that pyruvate kinase is essential for multicellular development and contributes to sporulation.

# B33

## DEVELOPMENTAL CHARACTERIZATION OF THE MYXOCOCCUS XANTHUS NLA18 MUTANT REVEALS A DEFECT IN PPGPP ACCUMULATION

***M. E. Diodati[1]****, F. Ossa[2], N. B. Caberoy[2], I. R. Jose[1], A. G. Garza[2], M. H. Singer[1];*
*[1]UC Davis, Davis, CA, [2]Syracuse University, Syracuse, NY*

When *Myxococcus xanthus* cells accumulate the intracellular starvation signal (p)ppGpp during amino acid starvation, they initiate a multicellular developmental process that yields spore-filled fruiting bodies. We are interested in understanding the pathway between the accumulation of (p)ppGpp and the expression of the earliest developmental genes, and identifying key players involved in the cell's decision to undergo the vegetative growth to development transition in response to starvation.
The earliest of the (p)ppGpp-dependent genes share a common regulatory feature, a $\sigma^{54}$-like promoter. These types of promoters require positive activation by an NtrC type of activator protein.
Characterization of insertion mutants in 28 putative *ntrC*-like activator genes (*nla* genes) and analysis of their developmental phenotypes, identified an *nla* mutant with strong vegetative and developmental defects (Caberoy, et al. 2003). Designated *nla18*, this mutant had a generation time of 10.5-16 hours compared to 5 hours for wild type cells, and exhibited a delayed and incomplete developmental phenotype.
Based on both β-galactosidase assays and RNA slot blot analyses, the *nla18* mutants are defective in the expression of early developmental *Tn5lac* transcriptional fusions, including *sdeK* (Ω4408) and *spi* (Ω4521). In addition, *nla18* cells have a partial defect for the production of the relA-dependent population density signal, A-signal. Using ppGpp accumulation assays with isogenic wildtype and *nla18* cells, we show that the levels of ppGpp in *nla18* cells are about 50% of wild type during vegetative growth and about 18% of wild type under starvation conditions. We propose that, the relatively low levels of ppGpp in the *nla18* mutant lead to the observed defects in fruiting body formation, cell-cell signal production, and developmental gene expression. Currently, vegetative microarray analyses are underway and may yield clues to Nla18's specific targets and its inability to initiate an appropriate starvation response.

## B34

### THE HOLDFAST-ASSOCIATED (HFA) PROTEINS OF *CAULOBACTER CRESCENTUS* AND THEIR ROLE IN HOLDFAST-MEDIATED ADHERENCE

***G. G. Hardy**, R. C. Allen, J. L. Cole, Y. V. Brun;*
*Indiana University, Bloomington, IN*

Attachment is essential for microorganisms to establish interactions with biotic and abiotic surfaces. *Caulobacter crescentus* offers a powerful experimental system to study the molecular mechanisms and regulation of adherence due to the detailed knowledge of relevant developmental processes in this organism. *C. crescentus* has a dimorphic life cycle generating both a sessile, stalked cell and a motile swarmer cell. Stable attachment of *Caulobacter* stalked cells to surfaces requires an adhesive holdfast comprised of both protein and polysaccharide. Several genetic loci have been identified that are involved in holfast-mediated adherence, but there is little information on the physical mechanism resulting in stable attachment to surfaces. Previous studies identified the holdfast associated (Hfa) proteins and demonstrated that mutations in *hfaA*, *hfaB* or *hfaD* result in holdfast shedding and decreased adherence. Our current work is focused on determining the role of the Hfa proteins in holdfast function. HfaA has similarity to fimbrial family proteins. HfaA is SDS- and heat-resistant and migrates as an aggregate unless formic acid denatured. We expressed an HfaA-M2 fusion protein in *C. crescentus* and Western blot analysis revealed that HfaA was expressed as an 11 kDa monomer in the outer membrane. Immunofluorescence localization indicates that both HfaA and HfaD localize to the stalk pole in predivisional and swarmer cells and to the tip of the stalk in predivisional and stalked cells. Yeast two-hybrid analysis of the Hfa proteins suggests that HfaA has a strong interaction with itself and possibly HfaD. All these data suggest that the Hfa proteins are important for the holdfast polysaccharide to associate with the tip of the stalk. We hypothesize that the Hfa proteins are involved in the formation of a fimbrial structure(s) at the tip of the stalk involved in anchoring the holdfast polysaccharide to the cell. These fimbriae would be composed of HfaA. HfaD is a membrane protein with limited similarity to the surface array protein RsaA and may act as a nucleator for the HfaA fimbriae. HfaA and HfaD would be translocated to the cell surface via the Sec pathway and then HfaB, a lipoprotein with similarity to the *E. coli* CsgG curlin usher.

## B35

### IDENTIFICATION OF AN ADDITIONAL GERMINATION GENE OF *BACILLUS SUBTILIS*

***C. C. Ferguson**, R. Losick;*
*Harvard University, Cambridge, MA*

Spores of *Bacillus subtilis* can lie dormant, resistant to a variety of environmental stresses, for extremely long periods of time. Given favorable conditions, however, a spore can rapidly germinate and develop into a metabolically active, growing vegetative cell. We have discovered a previously uncharacterized gene, which we call *gerT*, in which mutations cause a conspicuous defect in germination in response to at least two different germinants. *gerT* is an additional member of the regulon of genes under the control of the late sporulation regulatory protein $\sigma^K$ that was overlooked in previous transcriptional profiling analyses. Furthermore, *gerT* is repressed by GerE, a transcription factor acting at the terminal stage of the sporulation process and required for proper spore coat assembly. Consistent with its pattern of regulation, a GerT-GFP protein fusion localizes around the fully developed spore in a pattern typical of a coat protein and in a manner dependent on the spore structural protein SpoIVA. GerT-GFP also shows a surprising and atypical partial dependence on the outer coat protein CotE. GerT is not homologous to any known germination genes, and it may play a novel role in this poorly understood process.

## B36

### IDENTIFICATION OF A DEDICATED CHECKPOINT SYSTEM REQUIRED FOR INHIBITING CELL DIVISION AFTER DNA DAMAGE IN *CAULOBACTER CRESCENTUS*

***J. Spangler**, M. Laub;*
*Harvard University, Cambridge, MA*

Proper cell cycle progression requires the precise coordination of DNA replication and cell division. In response to DNA damage, cell division must be inhibited until after the DNA has been repaired and replication completed. Failure to inhibit cell division after DNA damage can have lethal consequences. In most prokaryotes, the molecular mechanisms that control cell division in response to DNA damage remain poorly understood. *Caulobacter crescentus*, a highly tractable microorganism, has become a model for the study of bacterial cell cycle regulation. *Caulobacter* cells are easily synchronized and cell cycle progression is easily tracked, both visually and by gene expression profiling. Cell cycle progression is accompanied by a series of morphological transitions. A

motile, G1-phased swarmer cell first differentiates into a sessile stalked cell, which then enters S-phase and initiates single round of DNA replication. After completion of DNA replication and chromosome segregation, the predivisional cell divides asymmetrically to produce a stalked cell, which is committed to an immediate round of DNA replication, and a swarmer cell, which must differentiate into a stalked cell prior to initiating DNA replication.
We have shown that DNA damage in *Caulobacter* induces a cell cycle checkpoint that delays cell division and causes cells to temporarily stall as pinched predivisionals. We have identified a previously uncharacterized gene, *cciA*, which is responsible for mediating this DNA-damage checkpoint. *cciA* was identified as a highly conserved member of the LexA regulon in *Caulobacter* and is rapidly up-regulated in response to DNA damage. *cciA* is completely dispensable under normal growth conditions, but in response to DNA damage, a Δ*cciA* strain does not properly delay cell division and hence produces inviable daughter cells. Furthermore, induced over-expression of CciA is sufficient to inhibit cell division in the absence of DNA damage. We have also shown that fluorescently-tagged CciA follows the same localization pattern as FtsZ, localizing to the septum in predivisional cells. This indicates that CciA may directly inhibit cell division by binding to a component of the cell division apparatus.
A checkpoint coupling DNA damage to cell division previously elucidated in *E. coli* is mediated by SulA, a damage-induced inhibitor of FtsZ polymerization. CciA, however, shows no homology to SulA and we present evidence that CciA inhibits cell division by a different molecular mechanism than SulA. Although non-homologous molecules and alternative mechanisms are employed, the regulatory strategy of a dedicated checkpoint system appears to be a widely conserved means of coupling DNA replication and cell division in bacteria.

# B37

## MOTILITY IN BACILLUS SUBTILIS IS GOVERNED BY A BISTABLE SWITCH

***D. B. Kearns***, *R. Losick;*
*Havard University, Cambridge, MA*

During the exponential phase of its life cycle, Bacillus subtilis grows either as single motile cells or as cells in long chains that are non-motile. Here we report that transition between the two growth states is biased by the swarming motility protein SwrA. The absence of swrA was found to promote growth in the chaining state whereas overexpression of swrA promoted growth as single, hyperflagellate motile cells. To explore the mechanism of SwrA, a functional SwrA-GFP fusion was found to localize to the cell nucleoid, suggesting a possible role in gene regulation. Transcriptional profiling revealed that SwrA activated a large number of genes (flagellar biosynthesis, chemotaxis, and autolysins) common to genes transcribed by RNA polymerase and the sigma factor, sigma D. However, fluorescence miscropcopy experiments with fusions to the gene for the Green Fluorescent Protein revealed that sigma D-controlled genes were active in single cells but were off in long chains. We propose that sigma D-directed gene expression is governed by a bistable switch and that SwrA functions to bias this switch toward the "on" state. We propose that cell chaining and motility are distinct developmental states that coexist during vegetative growth.

# B38

## TWO PHOSPHORELAY SYSTEMS CONTROL CELL DIVISION AND STALK BIOGENESIS IN *CAULOBACTER CRESCENTUS*

***E. G. Biondi***, *J. M. Skerker, M. Prasol, B. Perchuk, M. T. Laub;*
*Harvard University, Bauer Center for Genomics Research, Cambridge, MA*

Development and differentiation often involve the precise execution of major morphological changes at the cellular level. *Caulobacter crescentus* is an excellent model system in which to explore the molecular mechanisms responsible for temporal and spatial control of morphogenesis. In *Caulobacter*, cell cycle progression is accompanied by the precise execution, in time and space, of flagellum, pili, and stalk biogenesis. All of these morphogenetic events require the RNA polymerase $\sigma^{54}$ subunit RpoN. And as in other bacteria, to activate gene expression RpoN requires enhancer-binding proteins (EBPs) that typically must be phosphorylated by histidine kinases to stimulate transcription.
We show here that the EBP response regulator TacA is required for stalk biogenesis and proper cell division. We also identified a previously uncharacterized gene, *hptA*, with a similar deletion phenotype as *tacA*. The protein HptA has histidine phosphotransferase activity *in vitro*, suggesting that a multi-component phosphorelay activates TacA in *Caulobacter*. Using a novel, systematic biochemical assay, we examined the ability of HptA to phosphorylate each of the 43 response regulators encoded in the *Caulobacter* genome and found that HptA selectively passes phosphate to TacA. Using a similar systematic assay, we demonstrated that only two of the 27 hybrid kinases encoded in the *Caulobacter* genome, CC0138 and CC0921, can act as efficient phosphodonors for HptA *in vitro*. These results suggest that two phosphorelays, each utilizing HptA, lead to the phosphorylation of TacA. Phenotypic analysis of strains bearing deletions of each component (Δ*CC0138*, Δ*CC0921*, Δ*hptA*, and Δ*tacA*) supports the existence of these two phosphorelays *in vivo*. We further validate these pathways by demonstrating the ability of a constitutively active *tacA* allele, *tacAD54E*, to bypass deletions in components of the two phosphorelays. Finally, DNA microarray analysis of gene expression changes in the deletion mutants is presented to support the structure of these pathways *in vivo* and to identify potential target genes of TacA. In sum, we have shown that two phosphorelay systems in *Caulobacter* participate in the control of TacA activity, and hence stalk biogenesis. This work also demonstrates and validates the use of our novel, systematic biochemical

approach for the rapid and accurate delineation of multi-component signal transduction pathways. This approach can be adapted for the study of phosphorelay regulation in any organism.

# B39

## PROTEIN-PROTEIN INTERACTIONS IN ESCHERICHIA COLI CELL DIVISION MACHINERY

***D. LADANT**, G. KARIMOVA;*
*Institut Pasteur, PARIS, FRANCE*

Cell division in Escherichia coli is catalyzed by a number of essential proteins (named Fts) that assemble into a ring-like structure at the future division site. Several of these Fts proteins are intrinsic transmembrane proteins, whose functions are largely unknown. These proteins are recruited to the division site in a hierarchical order, initiated by the FtsZ protein, a functional analog of eukaryotic tubulin. However, the molecular interactions underlying the assembly of the septosome remain mainly unspecified. In this work, we have characterized interactions between the Fts transmembrane proteins by a bacterial two-hybrid approach that is based on interaction-mediated reconstitution of a cAMP-signaling cascade (Karimova et al., 1998, Proc. Natl. Acad. Sci. USA, 95 :5752). Our results revealed an extended network of interactions among the Fts proteins that are connected to one another through multiple interactions involving distinct polypeptide regions of each partners. Furthermore, we showed that the association between two Fts-hybrid proteins could be modulated by the co-expression of a third Fts partner. Altogether, these data suggest that the cell division machinery assembly is driven by the cooperative association between the different Fts proteins to form a dynamic multi-protein structure at the septum site.

# B40

## IDENTIFICATION OF POLYSTYRENE ADHESION MUTANTS IN *CAULOBACTER CRESCENTUS*

***C. E. Broderick**, M. Lawler, E. Toh, Y. Brun;*
*Indiana University, Bloomington, IN*

*Caulobacter crescentus* is a gram-negative dimorphic bacterium found in aquatic environments. *C. crescentus* undergoes programmed developmental changes that give rise to two different progeny cells at every cell division, a stalked cell and a motile swarmer cell. Stalked cells adhere to various surfaces through the adhesive holdfast, found at the tip of the stalk. The holdfast is made in part of a polysaccharide, composed of N-acetylglucosamine (NAG). In addition to the holdfast, pili and motility are also required for optimal adhesion to surfaces. To identify new components involved in the cell adhesion process, a screen was conducted for Mariner transposon mutants that were unable to bind to polystyrene microtiter plate wells. Mutants with adhesion defects were divided into various classes based on the presence of NAG at the tip of the stalk, formation of rosettes, motility, and the presence of pili. Many of the mutants had normal lectin staining of NAG, formed rosettes, synthesized pili, and had normal motility, indicating that the mutations identify new adhesion functions in *C.crescentus*. Insertions in several genes that resulted in an adhesion phenotype were identified. The mutations were mapped to three glycosyltransferase genes predicted to be involved in polysaccharide synthesis, two genes predicted to be involved in fatty acid metabolism, the response regulator *pleD*, and a gene predicted to encode a tryptophan halogenase involved in the chlorination of tryptophan. These results suggest that the holdfast polysaccharide contains sugars other than NAG and that non-sugar components, or modifications of the holdfast polysaccharide, may be important for surface adhesion.

# B41

## POST-TRANSCRIPTIONAL REGULATION OF SDA: A DEVELOPMENTAL CHECKPOINT PROTEIN IN *BACILLUS SUBTILIS*

***M. Ruvolo**, K. Mach, W. Burkholder;*
*Stanford University, Stanford, CA*

Sda is a small protein that establishes a developmental checkpoint inhibiting sporulation in *Bacillus subtilis* under conditions of DNA damage or blocks in replication. Cells can proceed into sporulation when these blocks are lifted and the chromosome has been replicated. For this to occur, the levels of Sda must be reduced. We are investigating the role of post-transciptional regulation in controlling Sda levels. Using pulse-chase analysis we have shown that Sda is an unstable protein with a half-life of four minutes, consistent with regulation by proteolysis. *B. subtilis* contains a family of AAA+-ATPase proteases that target substrates for degradation in a sequence specific manner. We have determined that one of these, the ClpXP protease, is involved in the proteolysis of Sda. The C-terminal sequence of Sda is important for efficient degradation. Replacing the two C-terminal serine residues with aspartic acids increases the stability of Sda. To determine whether Sda stability is regulated by replication stress, we assayed Sda stability when chromosome replication was perturbed. Replication stress did not affect Sda stability. Our results suggest that *sda* responds to perturbations in replication at the transcriptional level. The instability of Sda ensures that changes in the transcriptional rate of *sda* are rapidly mirrored at the protein level.

# B42

## MOLECULAR BASIS FOR CHROMOSOME COHESION IN SPORULATING CELLS OF *BACILLUS SUBTILIS*

***D. Claessen**, J. Errington;*
*University of Oxford, Oxford, UNITED KINGDOM*

Efficient segregation of chromosomes is crucially important in all living organisms. In eukaryotes, cohesion of the replicated chromosomes is an important step in this process, which occurs prior to attachment to the mitotic spindle apparatus. Chromosome cohesion also occurs in some prokaryotes. In sporulating cells of *Bacillus subtilis* the replicated sister chromosomes adopt an unusual extended conformation, known as the axial filament, that reaches from one cell pole to the other. It is well-known that formation of the axial filament depends on anchoring of the replicated chromosomes to the cell poles. However, it also appears to involve cohesion between sister chromosomes at mid-cell. We are currently unraveling the mechanism by which the two sister chromosomes are held together. We will use two approaches for this problem. First, we will carefully analyze nucleoid morphology and the (dynamic) localization of a series of selected markers during axial filament formation and resolution, among which the chromosomal terminus region (*terC*) is the likely cohesion site. Second, we will test more specific mechanisms involved that could generate (and/or resolve) chromosome cohesion. Examples of such mechanisms are e.g. a block in the final stages of the replication process, formation of chromosome dimers or catanae, or specific protein-DNA interactions. Knowledge emerging from this research will deepen our understanding of chromosome segregation in *B. subtilis*, and more generally, will contribute to the fascinating question of how nucleoid architecture is coupled with cell division and development in this organism.

# B43

## HELICAL AND BAND-LIKE LOCALIZATION OF PROTEINS INVOLVED IN NUTRIENT UPTAKE IN *CAULOBACTER CRESCENTUS*

***J. K. Wagner**, Y. V. Brun;*
*Indiana University, Bloomington, IN*

We investigated the pattern of outer membrane protein synthesis in *C. crescentus* and found that new outer membrane material is incorporated in a band-like or helical pattern. We also examined the localization of several proteins involved in nutrient uptake including ExbB and a component of the high affinity phosphate transport system, PstA. Interestingly, these proteins also localize in a pattern resembling spirals or bands. We tested the dependence of the patterns on the target of A22, an antibiotic which is proposed to inhibit the protein MreB. ExbB remains membrane-associated, but appears to become more diffuse after several hours of growth in the presence of A22. A similar result is seen in PstA localization following A22 treatment. Since A22 is believed to disperse MreB filaments after only minutes, these results suggest that ExbB and PstA maintain their band-like/helical arrangement even after MreB filament disassembly, but growth in the absence of MreB eventually leads to a change in their cellular targeting.

# B44

## OVEREXPRESSION OF THE TRANSCRIPTION FACTOR TACA INHIBITS MOTILITY OF CAULOBACTER CRESCENTUS

***M. V. Marques**, L. M. Menezes;*
*Inst. Ciencias Biomedicas, Univ. of Sao Paulo, Sao Paulo, SP, BRAZIL*

The tacA gene of Caulobacter crescentus encodes a putative sigma 54-activator whose expression is developmentally regulated during the cell cycle, being absent from the stalked cells and showing maximal levels of expression in the predivisional cells. A null tacA mutant was constructed by insertion of a spectinomycin resistance cassette, and this strain was shown to be motile, indicating that this factor is not essential for motility. A plasmid containing the tacA gene under control of an inducible xylose promoter was inserted into the tacA mutant strain, and motility was evaluated in a semisolid agar diffusion test. Normal motility was observed in medium containing glucose, but in the presence of xylose the cells presented no motility, showing a diffusion halo similar to the non-motile control rpoN strain. These results suggested that overexpression of tacA caused loss of cell motility. Analysis of expression of two flagellar genes, fljK and fljN, was performed using transcriptional fusions to the lacZ gene, in conditions of absence or induced expression of TacA. Transcription activity was determined by assaying the activity of b-galactosidase, and it was observed that transcription of the fljK/lacZ fusion increased in the absence of TacA, and overexpression of TacA causes a decrease in its expression. In the case of fljN/lacZ fusion, expression was lower in the absence of TacA, but overexpression of TacA caused a further reduction in transcription. The results indicate that an unbalanced level of TacA in the cell may affect flagellar gene transcription.

# B45

## DIRECTED MOTILITY IN MYXOCOCCUS XANTHUS BIOFILMS: A STUDY OF MULTICELLULAR CHEMOTAXIS

***R. G. Taylor,*** *R. D. Welch;*
*Syracuse University, Syracuse, NY*

A biofilm of *Myxococcus xanthus* is a predatory monoculture called a swarm that feeds on prey bacteria by expanding out from the swarm edge. We have demonstrated that an expanding *M. xanthus* swarm can also hunt by altering the direction of expansion toward a nutrient source. It is well established that a swarm can sense and respond to its environment; *M. xanthus* is capable of changing behavior in response to chemical signals, and it can even respond to physical stress placed on agar growth media (a behavior called elasticotaxis). Using time-lapse videomicroscopy, we have quantified the dependence of swarm movement and expansion rate on the presence of a nutrient gradient. *M. xanthus* is capable of detecting and moving up a nutrient gradient, and this movement depends upon only one of the two genetically separable *M. xanthus* motility systems, the Adventurous (A) motility system. Furthermore, we have demonstrated that the rate of swarm expansion can be genetically separated from a swarm's ability to respond to a gradient, and that this response is under transcriptional control. Specifically, we have identified several NtrC-like activator genes that, when disrupted, produce a swarm phenotype that has a wild-type expansion rate, but that does not exhibit directed motility. We are currently using microarray analysis to identify the genes affected by these NtrC-like activators.

# B46

## THE MREB AND MIN CYTOSKELETAL-LIKE SYSTEMS PLAY INDEPENDENT ROLES IN PROKARYOTIC POLAR DIFFERENTIATION

***Y. Shih,*** *L. I. Rothfield;*
*University of Connecticut Health Center, Farmington, CT*

Establishment of an axis of cell polarity and differentiation of the cell poles is a fundamental aspect of cellular development in many organisms. We compared the effects of two bacterial cytoskeletal-like systems, the MreB and MinCDE systems, on these processes in Escherichia coli. MinD underwent pole-to-pole oscillation in 82% of the spherical delta mreB cells, indicating that the Min proteins are capable of establishing an axis of polarity, independent of the MreB cytoskeleton. We showed two MreB-dependent polarization in E. coli that include the aspartate chemoreceptor Tar and a protein fragment of the Shigella virulence protein IcsA. This is consistent with previous reports that the MreB system is required for polar targeting of a number of polar constituents in several bacterial species. Thus, the two bacterial cytoskeletal-like systems appear to act independently on different aspects of cell polarization. Successive septal FtsZ rings are normally placed in parallel to each other in wild type rod-shaped E. coli cells. However, the FtsZ rings and planes of septation were placed at approximately right angles during the successive division cycles in the spherical delta mreB cells. This resembles the pattern previously described in spherical E. coli rodA mutants, mecillinam-treated cells and some spherical bacteria such as Neisserria. The perpendicular altenation of the division planes in delta mreB cells was accompanied by perpendicular altenation of the MinD oscillation axis during successive division cycles. We conclude that the cues used to establish the Min oscillation axis is independent of the MreB cytoskeleton. The mechanism responsible for the different patterns in successive division cycles in the presence and absence of MreB remains to be established.

# B47

## COMBINING MESOSCALE ANNOTATIONS FOR MYXOCOCCUS XANTHUS

***G. Suen***[1]*, B. S. Srinivasan*[2]*, R. D. Welch*[1]*;*
[1]*Syracuse University, Syracuse, NY,* [2]*Stanford University, Stanford, CA*

With the completion of the *Myxococcus xanthus* genome, we have been investigating new techniques for the prediction of gene function. For example, we have developed phylogenomic mapping, a genome-scale method of visualizing groups of genes based on their coinheritance (Srinivasan *et al*., 2005). The concept is based on the premise that pairs of sequence-dissimilar genes that are consistently coinherited in the same sets of organisms are likely to be functionally linked. Relationships are visualized as a topographical map of gene clusters. Phylogenomics is a form of mesoscale annotation - an annotation that provides data on the possible interactions within a genome based on subsets of genes that work together. We are currently constructing other types of mesoscale annotation. For example, we have constructed a gene expression map, which clusters genes based on their expression profiles from microarray data (Kim *et al*., 2001). Currently, we are using both phylogenomics and coexpression to resolve the inherent limitations for each type of map. By using both maps together, we can further resolve the likely gene interaction partners identified by each map independently. Preliminary data indicate that using both maps together increases our ability to identify likely genes involved with *M. xanthus* motility.

References

1.
Srinivasan, B.S. et al. The Phylogenomic Map of *Myxococcus xanthus* represents Generalizable form of functional Genome Annotation. *Nature Biotechnology* (In Press).

2.
Kim, S.K. et al. A gene expression map for *Caenorhabditis elegans*. *Science* **293**, 2087-2092 (2001).

# B48

## CHARACTERIZATION OF THE RAMR REGULON: A KEY REGULATORY PROTEIN COORDINATING THE MORPHOLOGICAL DIFFERENTIATION OF STREPTOMYCES COELICOLOR

***S. San Paolo***;
*Biozentrum, Basel, SWITZERLAND*

Streptomyces coelicolor, a Gram positive soil eubacterium, undergoes a complex growth cycle in which morphological differentiation coincides with the activation of the orphan response regulator RamR and the biosynthesis of the hydrophobic peptide SapB. SapB is an 18 amino acid morphogen encoded by the ramS gene that induces aerial hyphae formation (AHF) and sporulation by breaking the aqueous tension at the surface of the vegetative mycelium. A ramR disrupted mutant is strongly delayed in AHF, while constitutive overexpression of ramR accelerates AHF in the wild type strain and restores sporulation in the developmentally defective bld mutants both by inducing constitutive expression of ramCSAB genes and production of SapB. In order to characterize the ramR regulon, we performed microarray and quantitative RT-PCR analysis with RNA extracted from S. coelicolor during the course of development. In addition to the ramCSAB genes, another group of ramR dependent genes (rdg) was identified. This cluster includes: rdgA and rdgB, which encode two subunits of an ABC transporter, rdgK, a putative histidine kinase, and rdgR, a ramR paralog. EMSA and DnaseI footprinting experiments confirm that RamR exerts its effect on rdgABKR transcription directly, by binding three sites in the rdgABKR upstream region. These data suggest that RamR plays its key regulatory role in development of S. coelicolor by directly controlling SapB production and the expression of a second gene cluster. The existence of a regulatory loop between the ramCSAB and rdgABKR loci is under investigation.

# B49

## THE USE OF FUNCTIONAL GENOMICS TO IDENTIFY GENES INVOLVED IN BIOFILM FORMATION UNDER NUTRIENT LIMITATION

***M. D. Lynch***, *P. D. Bevins, R. T. Gill;*
*University of Colorado @ Boulder, Boulder, CO*

The formation of biofilms is an important and complex developmental process in bacteria, having industrial and medical implications. We have utilized a genome wide microarray based technique termed Mixed Library Parallel Gene Trait Mapping, to identify genes conferring a biofilm phenotype in *Escherichia Coli* in minimal media. This approach involves i) growth selections on an equal population mixture of 5 different plasmid based genomic libraries of defined insert sizes, ii) microarray studies of enriched plasmid DNA, and iii) wavelet based multi-resolution analyses that precisely identify the relevant genetic elements. This approach allows for the identification of single open reading frames as well as larger fragments, such as operons, that alter the expression of a given phenotype. This method was used to identify genes involved in biofilm formation. Continuous chemostat cultures of a mixture of *E. Coli* genomic libraries were grown in minimal media. After 48 hours of growth biofilms were observed in the culture vessel and samples taken. Plasmid DNA isolated from these samples was hybridized to microarrays prior to a multi-resolution analysis. The analysis identified five regions of the genome of varying sizes that when kept at higher copy led to the formation of biofilms. These regions surround the open reading frames *adrA*, *yddV*, *ydeH*, *yeaP* and *yliF* all which contain a conserved GGDEF domain. These domains encode a diguanylate cyclase, which produces cyclic di-GMP, a second messenger, previously reported to be involved in biofilm formation and cellulose production. Further analysis demonstrated that clones containing these regions produce biofilms in a media dependent fashion, growing planktonically in rich media and forming biofilms only under amino acid limitation. Staining and growth assays reveal that these clones exhibit morphological and physiological changes when subjected to amino acid limitation, including changes in encapsulation, adherence, extracellular matrix production and biofilm growth. Theses studies indicate a role for these domains in either sensing or relaying nutrient stress signals that result in biofilm development. Future efforts are aimed towards understanding the mechanistic basis of these pathways.

# B50

## BIOFILM AND FRUITING BODY FORMATION IN *BACILLUS SUBTILIS*

***F. Chu***[1], *S. S. Branda*[2], *D. B. Kearns*[1], *R. Kolter*[2], *R. Losick*[1];
[1]*Harvard University, Cambridge, MA,* [2]*Harvard Medical School, Boston, MA*

Wild strains of *B. subtilis* form architecturally complex communities of cells with fruiting-body-like aerial structures in which spore formation takes place preferentially at the tips. The aggregation of cells into these complex communities (biofilms) appears to be mediated by the formation of long, filamentous bundles that are held together by an extracellular matrix. Here we present evidence that the master regulator for the formation of the extracellular matrix is the repressor protein SinR. We previously reported that SinR directly represses transcription from an operon consisting of 15 genes

believed to govern the biosynthesis of an exopolysaccharide component of the matrix. We now report the identification of an additional three-gene operon, *yqxM-sipW-tasA*, that is under the direct control of SinR and is required for the aggregation of cells into multicellular communities. The products, pre-YqxM and pre-TasA, encoded by the first and third members of the operon are secreted proteins that are processed under the direction of the dedicated signal peptidase SipW. Mutants lacking either secreted protein form aberrantly thin biofilms and flat, featureless colonies. An extracellular complementation experiment indicates that TasA acts in an extracellular manner to promote cell aggregation and is probably a component of the matrix, along with the expolysaccharides. We present biochemical and molecular genetic evidence that SinR binds to multiple sites in the promoter region of the operon to block its transcription. *In toto*, our results are consistent with the idea that SinR is a master regulator between a motile state in which cells are capable of swimming or swarming and a sessile state in which cells form complex multicellular communities. Progress in elucidating upstream components of the pathway controlling SinR-mediated repression will be presented.

# B51

## ROLE OF THE MEMBRANE TARGETING SEQUENCE IN MIND OSCILLATION

*T. H. Szeto, S. L. Rowland, L. Chen,* ***G. F. King****;*
*University of Connecticut Health Center, Farmington, CT*

MinD is an ATPase that plays a central role in the Min system that negatively regulates placement of the division septum in both Gram positive and Gram negative bacteria. It is the best conserved and most widely distributed of the Min proteins, being found in eubacteria, archaea, and plastids. Remarkably, however, the mechanism of MinD action is not conserved. In Escherichia coli, the protein undergoes a rapid pole-to-pole oscillation that lasts throughout the entire cell cycle, whereas in Bacillus subtilis it is tethered to the cell poles at all stages of development except very late during septation, when it is recruited to the division site. The reasons for such dramatic differences in the mechanism of MinD deployment are unclear. We recently demonstrated that membrane localization of MinD is mediated by an 8-12-residue C-terminal motif termed the membrane targeting sequence or MTS [Szeto et al. (2002) Proc. Natl. Acad. Sci. USA 99, 15693-15698]. We further demonstrated that the MinD MTS interacts directly with lipid bilayers as an amphipathic helix, with a distinct preference for anionic phospholipids [Szeto et al. (2003) J. Biol. Chem. 278, 40050-40056]. However, the lipid preference and membrane affinity of the MTS differs significantly between organisms and appears to be causally related to whether MinD oscillates or not. We will present data on a range of native and mutant MTSs that support the cooperative polymerization or zipper model that we previously proposed to explain the reversible membrane association of E. coli MinD during its pole-to-pole oscillation cycle.

# B52

## A MODEL FOR REGULATION OF FTSZ ASSEMBLY IN *BACILLUS SUBTILIS* BY THE DIVISION INHIBITOR EZRA

***D. P. Haeusser****, P. A. Levin;*
*Washington University in St. Louis, Saint Louis, MO*

In response to an unidentified cellular signal, the prokaryotic tubulin homolog FtsZ assembles into a medial ring structure that serves as the base for the bacterial division machinery. Like tubulin, FtsZ assembles *in vitro* into single stranded filaments and bundles in the presence of GTP. *In vivo*, the precise timing and placement of FtsZ assembly is critical to ensuring that division occurs at the proper location and only once per cell cycle. This exquisite control is achieved through the actions of several proteins that regulate FtsZ assembly dynamics. One such factor is EzrA, an ~65 KDa membrane-associated protein present in low GC Gram positive bacteria. A null mutation in *ezrA* leads to the formation of extra FtsZ rings at the poles of *Bacillus subtilis* cells, suggesting that EzrA normally functions to prevent aberrant FtsZ assembly. *In vitro*, a purified EzrA fusion protein interacts directly with FtsZ to block FtsZ assembly, but is unable to disassemble preformed FtsZ polymers. Although GTPase data indicates that EzrA is not a monomer-sequestering protein, we have yet to determine the mechanism by which EzrA inhibits FtsZ assembly. In an effort to enhance our understanding of the FtsZ - EzrA interaction we have extended our biochemical experiments to test several models for EzrA-mediated inhibition of FtsZ assembly. In preliminary experiments, three different assays - 90 degree angle light scattering, electron microscopy, and FtsZ-GFP assembly reactions on glass slides - support a model in which EzrA 'caps' FtsZ polymers to prevent further assembly while also protecting preformed polymers from disassembly. Future experiments are directed towards verifying our preliminary data and identifying the regions and residues responsible for the EzrA - FtsZ interaction.

# B53

## ANALYSIS OF TWO SERINE/THREONINE KINASES, PKTA5 AND PKTB8, REQUIRED FOR MYXOCOCCUS XANTHUS DEVELOPMENT

***E. Stein***[1]*, K. Cho*[2]*, P. Higgs*[1]*, D. Zusman*[1]*;*
[1]*UC-Berkeley, Berkeley, CA,* [2]*Hoseo University, Asan Chungnam, REPUBLIC OF KOREA*

**Poster Abstract**
Under starvation conditions, *Myxococcus xanthus* undergoes a carefully regulated developmental program which includes aggregation and sporulation. The *espAB* locus has been

implicated in the control of developmental timing. Deletion of *espA* causes premature cellular aggregation and sporulation by 16 hours compared to wildtype. Interestingly, *espA* mutants also sporulate outside of fruiting bodies. Deletion of *espB* delays aggregation by 16 hours and sporulation efficiency is reduced to below 30% of wildtype levels. *pktA5* and *pktB8,* which encode two putative serine/threonine kinases, flank the developmentally-regulated *espAB* operon. Deletion of either *pktA5* or *pktB8* gives a phenotype that resembles an *espB* mutant (delayed aggregation and reduced sporulation efficiency). Epistasis experiments revealed that *espA* is epistatic to *espB* and partially rescues a *pktA5* or *pktB8* mutant. Analysis of *lacZ* fusions revealed that *pktB8* is expressed during vegetative growth and is not expressed during development. In contrast, *pktA5* is only expressed during development. Our present studies are concerned with characterizing the putative serine/threonine kinases. We have cloned both *pktA5* and *pktB8* genes and studied their activities. *In vitro* phosphorylation assays showed that PktA5 can autophosphorylate, whereas a PktA5K74R point mutant that should be kinase deficient, cannot autophosphorylate. PktA5~P can also catalyze a phosphotransfer reaction to the artificial substrate, myelin basic protein *in vitro*. Experiments are currently underway to study the relationship between the serine/threonine kinases and the *espAB* operon and how they contribute to *M. xanthus* development.

# B54

## MUTATIONS IN *DIVL* AND *CCKA* SUPPRESS A *DIVJ* NULL MUTANT OF *CAULOBACTER CRESCENTUS*

***D. L. Pierce****, D. S. O'Donnol, R. C. Allen, J. W. Javens, Y. V. Brun;*
*Indiana University, Bloomington, IN*

The histidine kinase DivJ participates in a phosphorelay pathway controlling cell division and polar development in *Caulobacter crescentus*. Mutations in *divJ* lead to filamentous cells that produce long, often misplaced, stalks. We used a suppressor screen of a Δ*divJ* mutant's slow growth phenotype to identify other genes involved in the regulatory pathway. Twenty-seven independent suppressors of the *divJ* mutant were isolated. Sixteen suppressors map to the *pleC* region, two map to *cckA*, and three map to *divL*. Many of the *pleC* mutations are frameshifts that result in an early truncation of the protein, and others are substitutions near the catalytic histidine, which do not affect the length of the protein, but likely affect its ability to function. We expect all of the suppressor mutants in *pleC* to be null alleles. However, a constructed Δ*pleC* Δ*divJ* double mutant has a growth rate identical to that of the Δ*divJ* mutant, whereas the *divJ* suppressor strains with null mutations in *pleC* have a faster growth rate. Since a null mutation in *pleC* is not sufficient to suppress the growth rate defect of the *divJ* mutant, those suppressor strains must contain a second mutation that suppresses the growth rate defect of the *divJ* mutation. Data obtained during the mapping of the suppressor mutations suggests that the second mutation requires a null mutation in *pleC* in order to suppress the growth defect of *divJ* mutants and that the second mutation maps near *pleC*. The two suppressor mutations in *cckA* are single nucleotide substitutions which result in single amino acid substitutions. One suppressor mutation is just before the kinase domain and the other is in the kinase domain. Both mutations likely affect the ability of the protein to autophosphorylate. The three mutations in *divL* are all single nucleotide changes leading to single amino acid changes upstream of the kinase domain. The mutant alleles of *divL* confer a temperature sensitive (TS) phenotype. We hypothesize that the phenotype of the *divJ* mutant is due to an abnormally high level of CtrA activity. Indeed, the activity of the CtrA-dependent *pilA* promoter is higher than in wild-type cells in a Δ*divJ* mutant and is reduced in the suppressor strains as well as in otherwise wild-type strains harboring the *divL* suppressor alleles.

# B55

## ADVENTUROUS GLIDING MOTILITY OF MYXOCOCCUS XANTHUS REQUIRES A COILED-COIL PROTEIN AND A SMALL GTPASE

***P. L. Hartzell****, R. Yang, T. B. Plocher, S. Bartle;*
*Univ of Idaho, Moscow, ID*

Wild-type M. xanthus cells use two genetically independent motility systems - adventurous (A) and social (S) - to gliding over surfaces that are used simultaneously by wild-type cells. Although the Frz chemotaxis proteins are known to control cell reversal, it is unclear how coordination of the A and S motility engines is achieved during cell reversal. Genetic and biochemical data suggest that MglA coordinates the A and S motors. MglA is a small monomeric GTPase that is required for both A and S gliding movement. MglA interacts with at least two different proteins. One protein partner, MasK, is a membrane-bound tyrosine kinase that was identified in a suppressor search; a mutation in masK restores S motility to an mglA mutant. The second partner, AglZ, was identified from a yeast two-hybrid assay in which MglA was used as bait. Disruption or deletion of aglZ abolishes movement of isolated cells, showing that aglZ is part of the Adventurous gliding system. The aglZ gene encodes a 153kDa protein that has a N-terminal receiver domain characteristic of two-component response regulator proteins and a C-terminal coiled-coil domain that is similar to myosin. The coiled-coil domain of AglZ forms a regular striated-lattice structure when expressed in E. coli. Purified AglZ forms a filament structure in vitro and immunofluorescence suggests that the protein forms a cable-like structure in vivo.

# B56

## ROLE OF NOC IN SPORULATION OF *BACILLUS SUBTILIS*

***J. Schneeweiss;***
*University of Oxford, Oxford, UNITED KINGDOM*

Nucleoid occlusion protein Noc is a DNA binding protein and an inhibitor of cell division in *Bacillus subtilis*. In vegetative cells of *B. subtilis* it plays an important role in the control of division site placement, preventing septation through the nucleoid. Noc works in conjunction with the Min system, a polar complex of MinD, and the Z-ring inhibitor MinC which prevents septation at the cell poles. Combination of these two systems results in correct division site placement at midcell. By locating to the nucleoids and preventing septation there, Noc could also provide a mechanism for coordination of chromosome segregation and septation. A collection of mutant alleles of *noc* were studied for their effect on cell division in different genetic backgrounds and at least three classes of mutants were defined. During sporulataion the nucleoid undergoes a conformational change into an extended structure called an axial filament. This is followed by an unusual septation, which occurs close to the cell pole, instead of at midcell, as in vegetative growth. Overexpression of *noc* had previously been shown to have a negative effect on sporulation efficiency. We have analysed the sporulation defects of cells overproducing Noc, and determined that this effect was due to reduced levels of polar septation. Further analysis of the effects of *noc* deletion and overexpression will be presented.

# B57

## PARALLEL UNUSUAL TWO-COMPONENT SIGNAL TRANSDUCTION SYSTEMS REPRESS DEVELOPMENTAL PROGRESSION IN THE SOCIAL PROKARYOTE MYXOCOCCUS XANTHUS

***P. I. Higgs***[1]*, K. Cho*[2]*, D. E. Whitworth*[3]*, L. S. Evans*[3]*, D. R. Zusman*[1]*;*
*[1]University of California at Berkeley, Berkeley, CA, [2]Hoseo University, Asan, REPUBLIC OF KOREA, [3]University of Warwick, Warwick, UNITED KINGDOM*

Myxococcus xanthus is a social bacterium that undergoes a complex life cycle. Under starvation conditions, the bacteria initiate a developmental program in which groups of approximately 100,000 cells migrate into mounds (fruiting bodies) and then differentiate into environmentally-resistant myxospores. Previously, we demonstrated that deletion of espA, which encodes a sensor histidine kinase, results in a phenotype of accelerated development relative to the wild type strain. In our model, we envisioned that EspA functions to repress the progression of development until an unidentified signal is sensed. In a genetic screen designed to identify a cognate response regulator for EspA, we instead identified the red (regulation of early development) operon consisting of at least seven genes including four unusual two-component signal transduction (TCS) genes, redCDEF. espA, redCDEF double mutants display a strikingly additive developmental phenotype; double mutants sporulate at least 12 hours earlier than each single mutant and fail to form fruiting bodies. These results suggest that EspA and RedCDEF independently repress progression of development. However, genetic epistasis experiments and yeast two-hybrid interaction analyses suggest that the four Red TCS proteins do function together in a linear signal transduction pathway. Together, these results and data from other research groups, suggest that progression through the developmental pathway in M. xanthus is subject to control by multiple parallel sets of TCS. We are currently using genetic and biochemical techniques to further characterize the TCS systems identified in this study.

# B58

## ANALYSIS OF THE BINDING AFFINITY OF TWO TYPES OF L31 TO THE RIBOSOME IN *BACILLUS SUBTILIS*

***G. Akanuma****, Y. Natori, N. Nomura, H. Nanamiya, F. Kawamura;*
*Rikkyo University, Tokyo, JAPAN*

We have recently found that alternative localization of two types of L31 ribosomal protein, RpmE and YtiA, is controlled by intracellular concentration of zinc in *Bacillus subtilis* ribosome. However, very little is known about the function of these proteins as the ribosomal protein, and the biological significance of the regulatory mechanism. To obtain further information for the function of these proteins, we constructed an *rpmE* or a *ytiA* mutant and studied the mutational effect in detail. While the cellular level of RpmE in the wild type was decreased rapidly after the end of vegetative growth in CSM, it did not decrease in the *ytiA* disruptant. Next, we cloned the *ytiA* gene into downstream of the P*spac* promoter in the multicopy plasmid, pDG148, and studied the effect of *ytiA* overproduction on the intracellular level of RpmE. As the results, intracellular level of RpmE was apparently decreased when IPTG was added to the medium. Moreover, the half-life of RpmE turnover was 110.6 min with the addition of IPTG, while it was 1856.6 min without the addition of IPTG. These results suggested that the expression of *ytiA* affects the stability of RpmE in the cell. In addition, we found that the affinity for the ribosome of YtiA is higher than RpmE, and RpmE is expelled from ribosome by addition of YtiA, with *in vitro* ribosome reconstitution experiments. It is thus, most likely that the liberation of RpmE from the ribosome by YtiA results in the instability of RpmE.

# B59

## STATUS OF THE CHROMOSOME DURING DEVELOPMENT IN MYXOCOCCUS XANTHUS

***M. Singer***, *L. Tzeng;*
*Univ. of Califonia, Davis, CA*

The developmental process of Myxococcus xanthus is a differentiation process such that, a priori, no cell division events are required. We predict that it is possible that the cell cycle state of M. xanthus may play a role in the decision making process of whether to proceed with the developmental program. The goal of this project was to determine if cell cycle events govern progression through development. Vegetative cultures of M. xanthus are asynchronous with respect to the cell cycle, therefore, when development is initiated the population has a heterogeneous chromosome number. As a monitor of the cell cycle we have examined the chromosome content of vegetative M. xanthus cells and two developmental fates, myxosprores and peripheral rod cells, to determine the chromosome numbers of these populations. Using flow cytometry, fluorescence microscopy and FISH analysis, we have shown that myxospores and peripheral rod cells are homogeneous populations containing 2n and 1n chromosome states, respectively. These data strongly suggest that M. xanthus controls its cell division events during the developmental program. In addition, we have determined that DNA synthesis is required for entrance into the developmental program. Using DNA synthesis inhibitors we show that replication function is necessary for development up to 12 hrs, at which point cells become independent of DNA replication. Finally, using DNA microarrays, we report the state of expression of DNA repair, recombination and replication genes during development.

# B60

## BACTERIAL DNA SEGREGATION DYNAMICS MEDIATED BY PARF, A POLYMERIZING PROTEIN THAT ASSEMBLES INTO MULTISTRANDED FILAMENTS

***D. Barilla'***[1]*, M. F. Rosenberg*[1]*, U. Nobbmann*[2]*, F. Hayes*[1]*;*
[1]*University of Manchester, Manchester, UNITED KINGDOM,*
[2]*Malvern Instruments, Malvern, UNITED KINGDOM*

The partition cassette of plasmid TP228 includes two genes, parFG, and the upstream partition site, parH. The ParG protein binds to direct repeats in the parH site and recruits ParF onto the nucleoprotein partition complex. The ParF protein is a Walker-type ATPase of the ParA superfamily that assembles into extensive filaments in vitro. The polymerization process has been investigated by dynamic light scattering in parallel with sedimentation assays and the ultrastructure of the filaments has been analyzed by negative-stain electron microscopy. The fibres appear as multiple paired protofilaments with a more compact terminus at one end and a more frayed extremity at the opposite end. These filaments are remarkably similar to those observed for the evolutionarily-related MinD cell division protein, but are quite distinct from those described for the unrelated FtsZ tubulin homolog involved in cell division and the actin-type partition protein of plasmid R1. ParF polymerization is potentiated by ATP and inhibited byADP binding and it does not require nucleotide hydrolysis. The effect of mutations in two highly conserved residues of the Walker A motif has been investigated in vivo and in vitro: this mutational analysis has established a functional coupling between filament dynamics and proper DNA segregation. The partner partition protein ParG participates in the ParF polymerization process by playing at least two separable roles: it enhances ParF ATPase activity and promotes filament bundling. These results highlight another parallel with the MinDE system, as MinE also stimulates MinD ATP hydrolysis and reorganizes/remodels MinD filaments. The analogies observed between the ParFG and MinDE systems point to an evolutionary parallel between DNA segregation and cytokinesis in procaryotic cells and reveal a potential molecular mechanism for plasmid and chromosome segregation mediated by the ubiquitous ParA-type proteins. This work was supported by an MRC New Investigator Award to DB and by Biotechnology and Biological Sciences Research Council and Wellcome Trust grants to FH.

# B61

## PROTEIN LOCALIZATION STUDIES IN AERIAL HYPHAE OF *STREPTOMYCES COELICOLOR*

***S. H. Au-Young;***
*McMaster University, Hamilton, ON, CANADA*

*Streptomyces coelicolor* is a filamentous Gram-positive soil bacterium that is a model organism for secondary metabolism and multicellular differentiation. It produces specialized cell types: the substrate hyphae which synthesize antibiotics, and the aerial hyphae which metamorphose into chains of spores. Morphological development is governed by a number of different genes. The *ram* genes have been shown to be intimately involved in aerial hyphae formation and with the production of a small hydrophobic peptide, SapB which directs aerial hyphae to grow up into the air. Protein localization experiments with the *ramCSAB* operon using the enhanced green fluorescent protein and fluorescence microscopy are being conducted. Our results suggest a compelling model for the delivery of SapB to its likely site of action.

# B62

## A GENETIC SCREEN FOR THE SPOIIAB PROTEOLYSIS ADAPTOR IN BACILLUS SUBTILIS

***J. Kain;***
*Harvard University, Cambridge, MA*

The anti-sigma factor SpoIIAB holds SigmaF inactive until after the sporulation septum has formed in Bacillus subtilis. SpoIIAB is subject to proteolysis by ClpCP, contributing to the proper activation of SigmaF. The extreme C-terminal amino acids LCN of SpoIIAB constitute a necessary and sufficient degradation signal. Negative evidence from a number of experiments suggest that an adaptor protein may recognize the LCN tag and stimulate degradation of SpoIIAB via ClpCP. A genetic screen has been developed using GFP-LCN to identify the adaptor protein. Cells carrying the GFP-LCN reporter were mutagenized and screened for GFP stability. A number of mutants were found and are currently being mapped and characterized.

# B63

## MODEL OF AN OXYGEN-SENSING GENETIC NETWORK IN *CAULOBACTER CRESCENTUS*

***M. Tan****, S. Crosson, H. McAdams;*
*Stanford University, Stanford, CA*

A bacterial cell is exposed to a wide range of environmental conditions to which it must adapt in order to survive. We have studied the genetic regulatory circuit that controls the response of *Caulobacter crescentus* to varying levels of oxygen. The topology of the core circuitry, consisting of the sensor histidine kinase FixL, the cognate response regulator FixJ, the transcription factor FixK, and the kinase inhibitor FixT, is conserved between *Caulobacter* and rhizobial bacteria. This sensory signaling module is wired into both common and distinct downstream genes in the two species. We have constructed a mathematical model based on a set of ordinary differential equations in order to analyze the dynamics and input-output characteristics of the network. By exploring the parameter space and comparing with experimental evidence, we have shown that different parameter values (primarily kinetic constants) are found in *Caulobacter* and the rhizobia. We have also identified the subset of parameters critical in determining the switch-like output behavior of the system. In addition, our modeling results suggest that there might be additional, yet-to-be-identified components in the network topology.

# B64

## A CONSENSUS CHROMOSOME ORIGIN OF REPLICATION IN A-PROTEOBACTERIA

***S. M. Shaheen****, G. T. Marczynski;*
*McGill University, Montreal, PQ, CANADA*

*Caulobacter crescenuts* is a Gram negative bacterium with a defined cell cycle. It divides to yield two distincts cell types: a flagellated, non-replicating swarmer cell and a non- motile but replicative stalked cell. In order to initiate chromosome replication, the swarmer cell must differentiate into the stalked cell by ejecting the polar flagellum and acquiring a tubular stalk appendage. Therefore, chromosome replication is restricted to the stalked cell where chromosome replication initiates exactly once per cell cycle. A key feature of *C. crescentus* is the global response regulator CtrA that controls key events within the cell cycle including chromosomal replication.
CtrA represses replication in the swarmer cell by binding to five specific sites on the *C. crescentus* chromosome origin of replication (*Cori)*. In order to allow replication to initiate, CtrA is removed from the stalked cell by proteolytic degradation. *C. crescentus* represents the first example where a response regulator protein controls DNA synthesis.We tested whether other replication origins from related bacteria also bind CtrA, and whether they also share other DNA essential elements implicated in replication control such as binding sites for the DnaA protein (DnaA boxes) and DNA methylation sites. We isolated potential replication origins from closely related freshwater and marine *Caulobacter* species by PCR cloning with degenerated primers that hybridize to a conserved gene cluster. Further, we wanted to know whether we amplified the true origin of replication for the related bacteria or not. Therefore we tested these clones by autonomous replication assays in *C. crescentus.* We identified origins of replication for 3 freshwater and 2 marine *Caulobacter* species, and sequenced all of them. 4 out of 5 clones supported autonomous plasmid replication in *C.crescentus.*The exception was the most distantly related marine species (MCS18). Based on sequence information and autonomous plasmid replication, we reveal that freshwater and marine *Caulobacter* origins have both common and unique properties such as an AT- rich region, and DnaA boxes, as well as unique *C.crescentus* features such as CtrA binding sites and CcrM DNA methylation site. We have also tested whether CtrA and DnaA bind with (MCS10) DNA in *vitro*, although its origin is most distantly related to *Cori.* In this footprint assays we observed that both CtrA and DnaA proteins bind in *vitro.* We further performed site directed mutagenesis in CtrA site e for MCS 10 to know the replicative ability of mutated DNA.We observed mutated DNA failed to replicate autonomously. We conclude that the unusual replication origin gene cluster and the response regulator CtrA appear to be conserved within the α-proteobacteria.

# B65

## USING PHYLOGENOMIC ANALYSIS TO IDENTIFY NEW GENES THAT ARE REQUIRED FOR MOTILITY AND FRUITING BODY FORMATION IN MYXOCOCCUS XANTHUS

***K. A. Murphy***[1]*, B. S. Srinivasan*[2]*, N. B. Caberoy*[1]*, G. Suen*[1]*, R. G. Taylor*[1]*, R. Shah*[1]*, F. Tengra*[1]*, B. S. Goldman*[3]*, R. D. Welch*[1]*, A. G. Garza*[1]*;*
*[1]Syracuse University, Syracuse, NY, [2]Stanford University, Stanford, CA, [3]Monsanto Corporation, St. Louis, MO*

When deprived of nutrients, *M. xanthus* initiates a complex developmental program that allows large groups of cells to migrate to aggregation centers and begin building multicellular fruiting bodies. Once a fruiting body is molded into its final shape, individual rod-shaped cells within this structure differentiate into dormant, spherical-shaped spores that are resistant to many forms of environmental stress. Due to the availability of the *M. xanthus* genome sequence, Srinivasan et al. (2005) were able to develop a phylogenomic map that clusters genes based on co-inheritance. We have been using the phylogenomic map to identify new genes that are required for fruiting body development. We focused on several clusters of putative motility genes, since motility is known to be required for fruiting body development. Plasmid insertions were made in 15 of these genes, and 12 of these insertion mutations affect motility and fruiting body development. The remaining genes in the motility clusters have been inactivated by plasmid insertion, and we are currently examining how these mutations affect motility and fruiting body development.

# B66

## FTSZ, THE NUCLEOID, AND MIND LOCALIZE IN PATTERNS THAT ACCOMMODATE PERPENDICULAR AND ALTERNATING CELL DIVISION PATTERNS IN GRAM NEGATIVE COCCI

*J. Szeto,* ***N. F. Eng****, J. R. Dillon;*
*University of Ottawa, Ottawa, ON, CANADA*

Faithful cell division in bacteria requires the placement of a contractile FtsZ ring at the midcell of rod-shaped bacteria. To assist in its proper positioning, the bacterial Min protein system in Gram-negative bacteria, such as the coccus *Neisseria gonorrhoeae* and the rod *Escherichia coli*, and the nucleoid are involved. Targeting FtsZ to the midcell is only achieved when MinC, MinD, and MinE work dynamically in concert to depolymerize FtsZ at the cell poles only, leaving the midcell free for FtsZ recruitment, polymerization and subsequent cell division initiation. The nucleoid itself also assists in this process by presumably preventing cell division across unreplicated and/or unsegregated DNA masses at midcell. Unlike cell division in rod-shaped bacteria, which occurs in parallel planes, the coccus *N. gonorrhoeae* exhibits cell division along two perpendicular, alternating planes to produce a 2 X 2 tetrad arrangement of daughter cells. This division pattern arrangement also occurs in round *E. coli* that lack *rodA* expression. Very few studies have attempted to address the mechanisms responsible for this distinct cytokinetic pattern. This study examined how the localization of FtsZ, the nucleoid, and MinD in Gram-negative cocci contribute to the septation pattern inherent to these round cells. We show that the nucleation point for new FtsZ assembly, following the first gonococcal cell division event, occurs at the junction point between two daughter cells, consistent with driving septation along a perpendicular plane. FtsZ localization also seems to be facilitated by the presence of the nucleoid. DAPI staining of the gonococcal nucleoid indicated that chromosomal DNA was not present in the vicinity of the two leading edges of constriction that specify the second perpendicular division plane. This is consistent with allowing FtsZ assembly to initiate in this region. Finally, we show that GFP-tagged gonococcal MinD not only migrates from pole-to-pole in round *E. coli*, but also traverses along the polymeric coiled array that winds around the longitudinal axis of dividing, round *E. coli*. These studies show that the localization patterns of FtsZ, the nucleoid and MinD, are arranged in a manner that would allow for the septation pattern that give rise to the tetrad phenotype inherent to *N. gonorrhoeae* and round *E. coli*.

# B67

## MULTIPLE RECEIVER DOMAINS REGULATE CELLULAR REVERSAL FREQUENCY IN *MYXOCOCCUS XANTHUS*

***Y. F. Inclan****, D. Zusman;*
*Univ of California Berkeley, Berkeley, CA*

*Myxococcus xanthus* utilizes two motility mechanisms to respond to environmental and self-generated cues and move on a solid surface. Pili pull cells by the leading pole while the adventurous motility motor(s) push cells from the lagging pole via a proposed slime secretion mechanism. Directional motility is achieved in part by the Frz signal transduction pathway which regulates the reversal period of cells. Frz proteins are homologs of enteric chemotaxis proteins and the Frz core comprises; a receptor FrzCD, a histidine protein kinase domain FrzE, and a coupling protein FrzA. At least three receiver domains are associated with the Frz pathway; one receiver domain fused to the histidine protein kinase domain of FrzE and two tandem receiver domains comprising FrzZ. A deletion of any core domain leads to hyporeversing cells. Deletion of the FrzE receiver domain leads to differential motility effects such that pilus mediated motility hyporeverses and adventurous motility hyperreveses (1). Deletion of either

FrzZ or all three receiver domains results in hyporeversing with respect to both motility systems, suggesting that FrzZ acts downstream of FrzE and strongly affects adventurous motility. How does the phosphorylation state of the receiver domains affect the motility systems? The kinase domain of FrzE acts as a phosphate donor to the receiver domain of FrzE (2) and the same is not known for FrzZ. I am currently using a genetic approach to better elucidate the motility role(s) of these receiver domains and a complementary biochemical approach to demonstrate phosphotransfer from FrzE to FrzZ.
1. Li *et al.* **J Bac** Mar 2005. p1716-1723
2. Acuna *et al.* **FEBS Lett**. 1995 Jan 16;358(1):31-3

# B68

## UNRAVELING THE FUNCTIONAL REGIONS OF A *CAULOBACTER* POLAR DEVELOPMENT PROTEIN

***M. L. Lawler***, *D. E. Larson, A. J. Hinz, Y. V. Brun;*
*Indiana University, Bloomington, IN*

*Caulobacter crescentus* is an aquatic bacterium with a dimorphic lifestyle. Each cell division yields progeny with two different fates: a motile swarmer cell with pili and a single flagellum, and a sessile stalked cell with an adhesive holdfast at the tip of the stalk. Strict regulatory control, both spatially and temporally, is essential to coordinate the different development of these two cell types. One protein important to this process is the **p**olar **o**rganelle **d**evelopment (PodJ) protein. PodJ exists in two different forms during the cell cycle, a full-length form (PodJL) and a shortened form (PodJS), which is the result of proteolytic cleavage of the long form. Both forms have different cellular functions: PodJL is required for pili formation, while PodJS is essential for swarming motility and holdfast formation. Each form has a specific localization pattern, mediated by different regions of the protein. Proper localization of this protein is important, as PodJ is required for the localization of the histidine kinase PleC and the pilus assembly protein CpaE. By making a series of stop codon insertions and an internal deletion in *podJ*, we have been able to unravel the regions involved in these various functions of PodJ.

# B69

## APICAL COMPLEXES OF DIVIVA AND THEIR ROLE IN ESTABLISHING THE POLARISED GROWTH OF STREPTOMYCES COELICOLOR

*S. Wang,* ***K. Flärdh****;*
*Lund University, Lund, SWEDEN*

In contrast to the situation in most other bacteria, the Streptomyces cell wall is polymerised at one cell pole, the hyphal tip. This pronounced tip extension is analogous to apical growth of filamentous fungi. It is not clear how the bacterial cell wall synthesis machinery is recruited to and organised at the hyphal tips, and how cell shape is determined at these sites in streptomycetes. Interestingly, the cell poles (hyphal tips) at which apical growth occurs are not generated by cell division, and each hyphal tip is instead established de novo as a lateral branch or germ tube in a process that is independent of septation and FtsZ. We have previously shown that the S. coelicolor homologue of DivIVA has an important role in hyphal tip growth. It localises distinctively to hyphal tips and nascent lateral branches. DivIVA was essential for growth, and partial depletion produced a phenotype strikingly similar to tip growth or nuclear migration mutants in fungi. Also, overexpression had dramatic effects on shape determination, leading to conspicuous swollen and pear-shaped cells. We can now show that induction of divIVA overexpression in pre-formed hyphae, that already contained a the DivIVA-EGFP hybrid protein at hyphal tips, rapidly lead to reorganisation of the apical DivIVA assemblage and to formation of several new discrete foci of DivIVA along the lateral walls. At those new foci, apical growth was initiated and branch-like lateral outgrowths emerged. Thus, DivIVA not only targets tips and affects morphogenesis, but is also instrumental in establishment of new tips. Furthermore, we report that DivIVA oligomerises and forms large protein complexes both in vitro and in vivo. The nature, composition, and shape of these complexes have been investigated with several methods. Based on these observations, it can be suggested that DivIVA forms large apical protein complexes in Streptomyces that are reminiscent of a cytoskeletal element, and have an important role in re-orienting cell polarity upon branching and in establishing tip extension, presumably by recruiting the machinery for cell wall assembly to the hyphal tip.

# B70

## FUNCTIONAL CHARACTERIZATION OF BACILLUS SUBTILIS PENICILLIN-BINDING PROTEIN 1 DOMAINS

*A. C. Talu, L. Liu,* ***D. L. Popham****;*
*Virginia Tech, Blacksburg, VA*

Penicillin-binding proteins (PBPs) play essential roles in the final stages of synthesis of the peptidoglycan layer of the bacterial cell wall. Sequence analysis and studies of enzymatic activities have revealed four domains in Class A PBPs, the major vegetative peptidoglycan synthetic enzymes. The N-terminal S-domain contains a cytoplasmic tail and an uncleaved signal peptide/membrane anchor. The N- and P-domains carry the glycosyl transferase and transpeptidase activities, respectively. The less-conserved C-terminal domain has no identified function. Loss of *Bacillus subtilis* PBP1 affects colony morphology, growth rate, cell length, cell diameter, regularity of wall thickness, and the degree of peptidoglycan

cross-linking. In order to examine the roles of PBP1 domains in affecting these various cell and protein properties, strains producing truncated and altered forms of PBP1 were constructed. The stability and the expression levels of all forms of PBP1 were examined. Mutant strain phenotypic properties studied include cell length and diameter, growth rate, and peptidoglycan structure. The data indicate that the PBP1 S- and P-domains do not play important roles in determining growth rate or cell shape, though the P-domain did have a significant effect on peptidoglycan cross-linking. Loss of function of the N-domain resulted in growth and morphology defects greater than those of a strain completely lacking PBP1. Mutants that are altered in one or more of these phenotypic properties allow us to associate functions with specific domains of PBP1.
We hypothesize that *B. subtilis* PBP1, interacts with other PBPs that function in modulating PG cross-linking, with autolysins, and with cytoskeletal proteins such as FtsZ and MreB, in order to produce a regular cell wall structure. We constructed a strain that produces PBP1a tagged with a C-terminal FLAG peptide epitope. Western blotting using anti-FLAG monoclonal antibodies showed that the anti-FLAG Abs could specifically recognize PBP1a-FLAG in membrane preparations. Immunoprecipitation was carried out using detergent-solubilized membrane proteins and agarose resin-bound monoclonal anti-FLAG antibodies. Immunoprecipitated proteins were separated by SDS-PAGE, and PBPs were visualized using $^{125}$I-penicillin-X. PBPs 1b, 3, 4 and 5 co-immunoprecipitated with PBP1a-FLAG, suggesting their potential contacts with PBP1a. We are using mass spectrometry to identify additional components of the peptidoglycan synthetic complex.

# B71

## PURIFICATION AND STRUCTURAL DETERMINATION OF SAPT, A LANTIBIOTIC-LIKE PEPTIDE INVOLVED IN AERIAL HYPHAE FORMATION IN THE STREPTOMYCETES

*S. Kodani[1], M. C. Durrant[2],* ***J. M. Willey[1];***
*[1]Hofstra University, Hempstead, NY, [2]John Innes Centre, Norwich, UNITED KINGDOM*

The streptomycetes are soil dwelling gram-positive bacteria that have a complex life cycle that includes the development of an aerial mycelium and spores. In *Streptomyces ceolicolor*, the formation of aerial hyphae involves the secretion of the hydrophobic peptide, SapB, which functions to release the surface tension at the colony/air interface. We recently showed that SapB has an unusual lantibiotic-like structure, although it apparently lacks antimicrobial activity. Because it appears that aerial hyphae cannot escape the aqueous milieu of the colony without biosurfactant activity, we postulated that SapB-like molecules may be produced by other *Streptomyces* species. To explore this possibility we isolated a peptide from *S. tendae* Tü901/8c, whose exogenous application rescued developmentally blocked streptomycete mutants. Chemical analyses of the peptide including Edman degradation after chemical modification, TOF/MS-MS and NMR were performed. The peptide consists of 21 amino acids and has a molecular weight of 2032 Da. We found that this peptide, which we call SapT, is also a lantibiotic. Unlike SapB with its two lanthionine bridges, SapT has 4 smaller loops as a result of three Me-lanthionine bridges and one lanthionine bridge. Like SapB, SapT is also very hydrophobic. Surprisingly, the *S. ceolicolor ramS* null mutant (*ramS* encodes the SapB prepeptide) treated with either SapT or SapB completed its developmental program, making not just aerial hyphae, but spores as well. This suggests that the previous assertion that SapB functions strictly as a biosurfactant may be inaccurate. Instead, SapB and SapT may have a role in signaling morphogenesis.

# B72

## CHARACTERIZATION OF FTSA MUTANTS AFFECTED IN TARGETING TO THE Z-RING

***S. Pichoff****, J. Lutkenhaus;*
*University of Kansas Medical Center, Kansas City, KS*

In *E coli*, the tubulin homolog FtsZ polymerizes to form the Z ring which is associated with the inside face of the cytoplasmic membrane at the division site. FtsA and ZipA interact directly with FtsZ and are the first known proteins recruited to the Z-ring. They are both required for the recruitment of later cell division proteins, however, either of them is sufficient to support Z-ring formation. In recent work we showed that FtsA, a protein similar to actin and other members of the ATPase super-family, can tether the Z ring to the membrane. FtsA's carboxy 15 amino acids are responsible for its association with the membrane and can form a putative amphipathic α helix. We had also shown that FtsA's association with the membrane is necessary for its targeting to the Z ring. We have suggested a model in which FtsA associates with the membrane before interacting with FtsZ and localizing to the septum. The interaction of FtsA with FtsZ will then allow FtsA to self interact. Using a combination of random and site-directed mutagenesis we have been able to identify mutations in *ftsA* that are probably impaired in FtsA interaction with FtsZ. The mutants are efficiently recruited to the membrane but not to the Z-ring. Overexpression of these mutants does not cause efficient filamentation, but formation of short chains of cells separated by deep indentations, as though cell division was impeded at a late stage of septation. Although these mutants still go to the membrane they do not interact with FtsZ or FtsA since they do not localize to the Z-ring. They may delay or disrupt cell division by inefficiently recruiting a later acting cell division protein away from the septum explaining the indentations. These mutations are

grouped in the same region on the FtsA structure and define a region on the surface of FtsA that interacts with FtsZ.

# B73

## MOLECULAR INTERACTIONS BETWEEN MREC, PENICILLIN-BINDING PROTEINS, AND OUTER MEMBRANE PROTEINS OF CAULOBACTER CRESENTUS

***J. W. Gober,*** *R. Ogorzalek Loo, J. A. Loo, A. V. Divakaruni; UCLA Dept. of Biochemistry, Los Angeles, CA*

Cellular morphology in prokaryotic organisms is dictated by the spatial pattern of peptidoglycan synthesis. Penicillin-binding proteins (PBPs), having glycosyl transferase and/or transpeptidase activities, catalyze the biosynthesis of this glycopeptide. The degradation and introduction of new murien strands required for cell elongation necessitates coordination between these enzymatic activities. To this end recent evidence has shown that these proteins do not function independently, but rather are present in a multi-enzyme complex(s). In Caulobacter crescentus, spatial and temporal organization of this complex has been shown to be dependent on the actin homolog MreB. We have previously shown that MreB is essential for viability. Depletion of mreB resulted in lemon-shaped cells that possessed cell wall integrity defects. MreB formed cell cycle-dependent spirals whose pattern changed in a fashion that paralleled the two morphogenetic phases of cell elongation and cell division. Early in the cell cycle, MreB spirals were distributed along longitudinal cell axis. In early predivisional cells, localization predominated at the midcell and did not disassemble until cell division initiation. In addition, PBPs also formed spiraled structures similar to, and dependent on, MreB. Downstream of MreB is the highly conserved cell shape-determining gene MreC. This protein is localized to the periplasm in all C. crescentus cell types forming helical patterns reminiscent of MreB and PBP2. MreC when covalently linked to CNBr-activated Sepharose specifically retained a number of penicillin-binding proteins (PBPs) from a crude Triton X-100 cellular extract. Also, several other non penicillin-binding proteins showed specific association with the MreC coupled sepharose compared to control column of Tris-coupled sepharose. Following excision from a polyacrylamide gel, these polypeptides were subjected to mass spectrometry-based protein identification. The isolated polypeptides contained a number of outer membrane and periplasmic proteins, a subset of which were found to be members of the TonB-dependent receptor family of proteins, previously implicated in the acquistion of nutrients. These receptors, as visualized by fusions to GFP, formed helical-appearing structures in the outer membrane. Similarly, the other retained proteins possessed spiral-like localization patterns. This localization patterning was also MreB dependent. Most of these MreC-interacting polypeptides were previously identified as enriched in proteomic analysis of the stalk, a cellular structure composed predominately of membrane and peptidoglycan. This data suggests that the internal cytoskeletal apparatus creates an internal spatial architecture that ultimately organizes components of the periplasm and outer-membrane.

# B74

## THE *CAULOBACTER CRESCENTUS FLAF* GENE ENCODES A NOVEL TRANS-ACTING FACTOR THAT COUPLES FLAGELLUM ASSEMBLY TO TRANSLATION

***J. W. Gober,*** *R. J. Dutton, J. Easter, Jr., M. Llewellyn; UCLA, Los Angeles, CA*

During each cell cycle of *Caulobacter crescentus* a sessile stalked cell undergoes asymmetric division to generate a flagellated swarmer cell. A complex, trans-acting hierarchy regulates the temporal expression of flagellar genes in the predivisional cell. For example, the expression and successful assembly of the flagellar hook structure is required for the translation of flagellins which comprise the filament. Previous experiments have demonstrated that FlbT operates a checkpoint coupling flagellin expression to the assembly of the hook-basal body structure. FlbT is known to post-transcriptionally repress 25 kDa flagellin (fljK) expression by destabilizing flagellin transcripts via an interaction with the 5' untranslated region (UTR). The mechanism by which completion of hook assembly is "sensed" by FlbT is not known. We now have evidence that the previously uncharacterized *flaF* gene in the *flbT* operon may encode a positive factor that antagonizes FlbT activity. The newly released genome sequences of several motile species of alpha-proteobacteria reveals that both *flbT* and a downstream gene, *flaF* are highly conserved and do not encode structural components of the flagellum. We created a mutant strain with a deletion in *flaF*. This strain was non-motile and failed to express any of the major flagellins as previously reported (Schoenlein and Ely, 1989). We have found that the flagellin *fljK* gene is transcribed in a *flaF* mutant, but is not translated. Introduction of a *flbT* mutation into this strain restored flagellin translation, but surprisingly did not restore motility. This result indicates that not only does FlaF serve to regulate flagellin expression, it is also required for flagellum assembly. Electron microscopy reveals that the flagellar filament is absent in *flbTflaF* mutant cells suggesting that FlaF is also required for secretion of flagellin monomers. One possibility is that FlaF functions as a flagellin chaperone, required for assembly and protein stability, however protein stability experiments indicate that flagellin is equally stable in a *flbTflaF* double mutant as it is in wild-type cells. Reporter assays demonstrate that *flbTflaF* exhibit a cell cycle pattern of transcription with a peak of activity in predivisional cells. Interestingly, the steady state levels of FlbT and FlaF exhibit markedly different expression patterns during the course of the cell cycle. While FlbT can be recovered equally from all cell types, FlaF is primarily present in predivisional cells during the time of filament assembly. Thus, it would appear that FlaF, in

response to both the progression of flagellar assembly and the cell cycle, provides a connection between translation and secretion of flagellin.

# B75

## UNDERSTANDING THE MECHANISM BY WHICH ABSA GLOBALLY REGULATES ANTIBIOTIC PRODUCTION IN *STREPTOMYCES COELICOLOR*

***Nancy L. Sheeler**, Justin R. Nodwell*
*McMaster University, Hamilton, Ont., Canada*

Streptomycetes are Gram-positive, soil dwelling bacteria that produce most of the antibiotics in clinical use, as well as numerous chemotherapeutic agents, immunosupressants and other drugs. *Streptomyces coelicolor*, a superior model organism for this genus, has been used extensively to understand prokaryotic differentiation as well as how antibiotic synthesis is regulated. The *S. coelicolor* life cycle is characterized by the generation of two distinct cell types: the antibiotic-producing substrate hyphae and the spore-forming aerial hyphae. *S. coelicolor* produces at least four antibiotics that are negatively regulated by the AbsA two-component signal transduction system. The *absA* locus encodes the sensor kinase AbsA1 and the response regulator AbsA2. We have shown that AbsA1 has both AbsA2 kinase and AbsA2~P phosphatase activity and that the phosphorylated form of the response regulator negatively inhibits antibiotic production. AbsA1 is predicted to have an unusual topology and signal-sensing domain. To understand the molecular mechanism of AbsA1 we need to identify which parts of the protein are intracellular and which are extracellular. Another unusual property of the AbsA two-component system is that it acts as a global negative regulator of antibiotic production even though its locus is embedded within one of the antibiotic clusters. The target promoters of AbsA2 and therefore the mechanism by which the AbsA system regulates antibiotic production is not known. Our current efforts are aimed at addressing these questions.

# B76

## ANTICO – *ASPERGILLUS NIGER* TRANSFER INTO COMMERCIAL ORGANISMS

***EMMA J. TILLEY**, I.S. HUNTER, ET AL* Department of Bioscience, University of Strathclyde, Royal College, Glasgow*

ANTICO is 5. Framework Programme project co-financed by the EU Commission. The programme will demonstrate that the transfer of specific genes from an *Aspergillus* strain, producer of citric acid, into other commercial microorganisms will enhance the pool of precursors in those organisms and lead to increased yields and production rates for their end products. The two key enzymes, 6-phosphofructo-1-kinase (pfk) and alternative oxidase (aox1) lead to increased glycolytic flux and the uncoupling the respiration from ATP production respectively, which, together with high glucose levels, will lead to increased metabolic fluxes and steady state levels of intermediates. The first task is to introduce the *A. niger* pfk gene, which was found to be up-regulated by phosphorylation, and the aox1 gene into the respective commercial strains, namely *Aspergillus terreus*, *Pichia pastoris*, *Lactococcus lactis*, *Streptomyces rimosus*, and *Streptomyces clavuligerus*. Such a demonstration of high yields and productivity will then be transferred directly to the production strains of European manufacturers.

# B77

## ISOLATION AND CHARACTERISATION OF STREPTOMYCETE STRAINS PRODUCING SIDEROPHORES FROM THAI AND BRITISH SOIL SAMPLES

*I. Nakouti[1], **E. Gaskell[1]**, D. Gilbey[1], P. Sihanoth[2], G. Hobbs[1]; [1]Liverpool John Moores University, Liverpool, UNITED KINGDOM, [2]Chulalonghorn University, Bangkok, THAILAND*

*Streptomyces* have developed an assortment of biochemical mechanisms to enable themselves to grow and survive in oligotrophic environments. Free iron is a particularly scarce entity in nature and these bacteria capture iron using an array of siderophores. Siderophores possess important pharmaceutical activity and in addition, have potential environmental applications in bioleaching. In an attempt to identify novel siderophore compounds we have isolated streptomycetes using selective isolation media from soil samples collected in Thailand and the UK. Siderophore production was screened using the CAS-assay system. Twenty Thai and five UK strains were confirmed CAS-assay positive exhibiting prolific siderophore production. The classes of siderophores expressed were determined using chemical assay procedures revealing that both hydroxamate and catechol type moieties were produced. A variety of morphological, cultural, physiological and biochemical characteristics were used to characterise the siderophore producers. Analysis of the nucleotide sequences of 16S rRNA was performed and we report on the taxonomic relationships of these isolates using PHYLIP. Interestingly most of these strains displayed pronounced bioactivity toward a range of microorganisms including yeast, fungi, gram +ve and gram-ve bacteria, suggesting that these organisms are highly adapted to competing in nature. We are currently optimising conditions for maximum biomass and siderophore production with a view to chemically characterising the siderophores.